THE USE OF WASTE MUSSEL SHELL IN SULFATE-REDUCING BIOREACTORS TREATING MINE-INFLUENCED WATERS

A thesis submitted in partial fulfilment of the requirements for the

Degree of Doctor of Philosophy

in Civil and Natural Resources Engineering

at the University of Canterbury

by

Benjamin Uster

Department of Civil and Natural Resources Engineering

University of Canterbury, New Zealand

September 2015

I grew up with an ambition and determination without which I would have been a good deal happier. I thought a lot and developed the faraway look of a dreamer, for it was always the distant heights which fascinated me and drew me to them in spirit. I was not sure what could be accomplished by means of tenacity and little else, but the target was set high and each rebuff only saw me more determined to see at least one major dream through to its fulfilment.

Earl Denman (1954)

ABSTRACT

Mining-Influenced Water (MIW) poses major environmental issues in New Zealand and worldwide due to a legacy of unmitigated mining activities. As conventional MIW treatment technologies can be very costly in terms of chemical and energy inputs, cheaper and environmentally-friendly alternative remediation strategies have been developed. These so-called passive treatment technologies include a range of engineered systems relying on biogeochemical processes able to mitigate the acidity and to immobilize the metals in MIW.

The present research, built on previous work conducted at the University of Canterbury, investigated the use of waste materials in mesocosm lab-scale sulfate-reducing bioreactors (SRBR) to treat actual mining-influenced water (MIW) sourced at an active coal mine in New Zealand. Specifically, this study investigated using waste mussel shells as an alkaline amendment (instead of the more conventional material limestone), with organic waste materials such as wood byproducts and compost in complex substrate mixtures in upward-flow SRBR. The influence of hydraulic retention times of approximately 3 and 10 days (HRT; i.e. the contact time between the MIW and the substrate mixtures in the SRBR) on the treatment performances was also evaluated.

Overall, each system successfully treated the MIW (e.g. increased the pH > 6 and removed >78 % of the metals, except Mn) during the first 5-month treatment period, while during the second 5-month period, the treatment systems containing limestone and/or operating at a short

HRT started to show signs of decreased efficiency. Generally, the system containing mussel shell and operating at a long HRT was constantly the most efficient system. Over the whole 41-week period of treatment, key metal removal efficiencies ranged between 97.6 and 99.7 % (Al), 83.9 and 95.2 % (Fe), and 9.2 and 38.8 % (Mn). Sulfate removal, in terms of moles of sulfate removed per cubic meter of substrate per day, was on average below the design values of 0.3 mol/m^3/d, and ranged between 0.03 and 0.55 mol/m^3/d (median values were 0.26 to 0.3 mol/m^3/d during the first 5-month period but dropped to 0.094 to 0.1 mol/m^3/d during the second 5-month treatment period).

The SRBR containing mussel shell instead of limestone resulted in significantly higher alkalinity generation (between 32 to 85 % higher) and higher metal removals (between 0.6 % higher for Al and 14 % higher for Ni). These results were mainly attributed to the unique mineralogy of the mussel shell which comprises of aragonite with traces of calcite, while limestone comprises of pure calcite with traces of quartz. The statistical analyses showed that the sulfate reduction was not significantly affected by the alkalinity source.

Similarly, systems operating at a longer HRT (10 days instead of 3 days) showed better treatment performances than systems operating at a short HRT in terms of alkalinity generation (44 to 62% higher), metal removal (between 0.5 % higher for Al to 15 % higher for Ni, and between 17 to 23 % higher for Mn), and sulfate reduction (50 to 77 % higher). Overall, the systems operation on a longer HRT were dominated by a more reduced environment facilitating the precipitation of metal sulfides, while the reactors running on a

shorter HRT were constantly maintained out of equilibrium by the continuous addition of fresh MIW.

Chemical and mineralogical analyses performed on the spent substrates suggested that the metals were removed through precipitation as, and adsorption onto, metal sulfides (Fe, Zn, Ni, Cu), (oxy)hydroxides (Al, Fe, Zn), and carbonates (Mn, Zn). Mn, a metal known to be harder to remove from solution was likely removed through the precipitation of rhodochrosite ($MnCO_3$) and via adsorption onto the organic matter. These results generally corroborated the results obtained using the geochemical modeling PHREEQC.

Overall, this study showed that mussel shells are not only a sustainable and effective alternative to mined limestone, but their use in SRBR would also result in a better treatment of MIW. Additionally, even though an increase in HRT resulted in a better contaminant removal, a HRT of approximately 3 days was sufficient to remove about 80% of all metals (except Mn). Therefore, the difficult choice of an optimal HRT must balance the need to meet a specific effluent quality while keeping the treatment time reasonably short, and an intermediate retention time of approximately 6 days could be optimal.

Deputy Vice-Chancellor's Office

Postgraduate Office

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

CO-AUTHORSHIP FORM

This form is to accompany the submission of any thesis that contains research reported in co-authored work that has been published, accepted for publication, or submitted for publication. A copy of this form should be included for each co-authored work that is included in the thesis. Completed forms should be included at the front (after the thesis abstract) of each copy of the thesis submitted for examination and library deposit.

Please indicate the chapter/section/pages of this thesis that are extracted from co-authored work and provide details of the publication or submission from the extract comes:

Partially reproduced in Chapters 2 and 6:

Uster, B., Trumm, D., Pope, J., Weber P., O'Sullivan, A.D., Weisener, C., Diloreto, Z.A. (2014) *Waste Mussel Shells to Treat Acid Mine Drainage: A New Zealand Initiative.* Reclamation Matters (Official publication of the American Society of Mining and Reclamation), Fall 2014, pp: 23-27.

(http://www.asmr.us/Publications/ReclamationMatters/2014/Fall2014Final.pdf)

Partially reproduced in Chapters 3 and 4:

Uster, B., O'Sullivan, A.D., Pope, J., and Trumm D. (2013) *Performance of sulphate-reducing bioreactors to treat AMD using waste mussel shells at different hydraulic retention times.* In Proceedings of the 2013 Australasian Institute of Mining and Metallurgy (AusIMM) New Zealand Branch Annual Conference, 25-28 Aug 2013, pp: 515-523 (best student presentation award).

Partially reproduced in Chapter 4:

Uster, B., O'Sullivan, A.D., Ko, S.Y., Evans, A., Pope, J., Trumm D., and Caruso B. (2014) *The use of mussel shells in upward-flow sulfate-reducing bioreactors treating acid mine drainage*. Mine Water and the Environment, Special Issue on Acid Mine Drainage in New Zealand. DOI: 10.1007/s10230-014-0289-1. Published online: 8th November 2014.

(http://link.springer.com/article/10.1007%2Fs10230-014-0289-1)

Partially reproduced in Chapter 5:

Uster, B., Milke, M., Webster-Brown, J.G., O'Sullivan, A.D., Pope, J., and Trumm, D. (2015) *Fe, Mn, and Zn removal and partitioning in sulfate-reducing bioreactors treating acid mine drainage* (in final stage preparation, to be submitted to Environmental Earth Sciences).

Please detail the nature and extent (%) of contribution by the candidate:

The candidate developed the methodologies (90%), carried out data collection (95%), data analyses (95%), and led manuscripts' writing (90%). Co-authors were mainly involved with interpretation of the results (still mostly the work of the candidate) and manuscript editing.

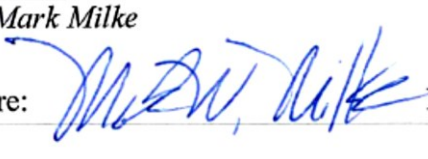
Certification by Co-authors:

If there is more than one co-author then a single co-author can sign on behalf of all.

The undersigned certifies that:

- The above statement correctly reflects the nature and extent of the PhD candidate's contribution to this co-authored work.
- In cases where the candidate was the lead author of the co-authored work he or she wrote the text.

Name: *Mark Milke*

Signature:  Date: 10 April 2015

ACKNOWLEDGMENTS

I would like to warmly thank a number of persons for their continuous help and support during those three years (and a few months). This work would not have been possible without their valuable knowledge and support. I am particularly thankful to the following people:

My numerous supervisors both at the University of Canterbury (UC) and at CRL Energy Ltd. (CRL): Aisling D. O'Sullivan (UC), Mark Milke (UC), Jenny Webster-Brown (UC), and Brian Caruso (UC), as well as James Pope (CRL) and Dave Trumm (CRL). They provided continual guidance, encouragements, and enthusiasm.

The technical staff at University of Canterbury, in particular: Peter McGuigan, Robert Stainthorpe, Matt Cockcroft, Mike Flaws, David MacPherson, Sally Gaw and Michael Sandridge. Their expertise and availability made the numerous hours spent in the laboratory almost painless. The staff at CRL Energy Ldt., especially Aaron Dutton, Mike Young, and Craig Fraser, and the staff at the mine for granting me access to their site.

I am also very grateful to have received the CRL Energy Postgraduate Scholarship (2012-2015), the University of Lausanne Young Researcher Grant (2012), and the American Society of Mining and Reclamation Ph.D. Memorial Scholarship (2014). It is needless to say that nothing could have happened without their financial support.

To all my new friends in New Zealand, with a special mention to Logan, Milad, and Laura. Thank you all for taking such good care of me, all the good moments spent with you will stay engraved in my memory forever.

And last, but certainly not least, my family for letting me follow my dreams. Especially, I would like to thanks my mom for the encouraging skype calls, messages, and the care packages. They meant more than can be expressed.

Thank you all!

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

AMD	Acid Mine Drainage
APHA	American Public Health Association
BOD	Biological Oxygen Demand
C/N	Carbon to Nitrogen ratio
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
EAS	Easily Available Substances
EDS	Energy Dispersive Spectroscopy
HDPE	High-Density Polyethylene
HRT	Hydraulic Retention Time
IC	Ion Chromatograph
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IQR	Interquartile Range
LOI	Loss on Ignition
LS-S	Limestone Short HRT system
LS-L	Limestone Long HRT system
MIW	Mining-Influenced Water
MS-S	Mussel Shell Short HRT system
MS-L	Mussel Shell Long HRT system
NIST	National Institute of Standards and Technology
ORP	Oxidation Reduction Potential
PP	Polypropylene
QA/QC	Quality Assurance/Quality Control
SEM	Scanning Electron Microscopy
SEP	Sequential Extraction Procedure
SI	Saturation Indices

SRB	Sulfate Reducing Bacteria
SRBR	Sulfate Reducing Bioreactor
TCLP	Toxicity Characteristic Leaching Procedure
TKN	Total Kjeldahl Nitrogen
TOC	Total Organic Carbon
TSS	Total Suspended Solids
TV	Trigger Values
VSS	Volatile Suspended Solids
XRD	X-Ray Diffraction
XRF	X-Ray Fluorescence

LIST OF PUBLICATIONS AND OUTCOMES

Peer-reviewed journal publications and conference proceedings:

Uster, B., Milke, M., Webster-Brown, J.G., O'Sullivan, A.D., Pope, J., and Trumm, D. (2015) *Fe, Mn, and Zn removal and partitioning in sulfate-reducing bioreactors treating acid mine drainage* (in final stage preparation, to be submitted to Environmental Earth Sciences).

Uster, B., O'Sullivan, A.D., Ko, S.Y., Evans, A., Pope, J., Trumm D., and Caruso B. (2014) *The use of mussel shells in upward-flow sulfate-reducing bioreactors treating acid mine drainage*. Mine Water and the Environment, Special Issue on Acid Mine Drainage in New Zealand. DOI: 10.1007/s10230-014-0289-1.

(http://link.springer.com/article/10.1007%2Fs10230-014-0289-1)

Uster, B., O'Sullivan, A.D., Pope, J., and Trumm D. (2013) *Performance of sulphate-reducing bioreactors to treat AMD using waste mussel shells at different hydraulic retention times.* In Proceedings of the 2013 Australasian Institute of Mining and Metallurgy (AusIMM) New Zealand Branch Annual Conference, 25-28 Aug 2013, pp. 515-523 (best student presentation award).

Conference abstracts and posters:

Uster, B., Milke, M., Webster-Brown, J.G., O'Sullivan, A.D., Pope, J., and Trumm, D. (2015) *Fe, Mn, and Zn removal and partitioning in sulfate-reducing bioreactors treating acid mine drainage*. 10th International Conference on Acid Rock Drainage (ICARD) & International Mine Water Association (IMWA) Annual Conference, Santiago, Chile. April 21-24, 2015 (poster).

Uster, B., Milke, M., O'Sullivan, A.D., Pope, J., Trumm, D., Caruso B. (2014) *The use of mussel shells in upward-flow sulfate-reducing bioreactors treating acid mine drainage.* 31st National Meeting of the American Society of Mining and Reclamation (ASMR), Oklahoma City, USA. June 14-18, 2014 (oral presentation, Honorable Mention).

Uster, B., O'Sullivan, A.D., Pope, J., and Trumm, D. (2013) *Performance of sulfate-reducing bioreactors treating mine influenced water using waste mussel shells.* 26th International Applied Geochemistry Symposium (IAGS), Rotorua, New Zealand, November 18-23, 2013 (oral presentation).

Others deliverables:

Uster, B (2015) *Sulfate reducing bioreactors treating mining-influenced water: investigating the influence of the hydraulic retention time and the alkalinity amendment.* Ph.D. Seminar, Department of Civil and Natural Engineering, University of Canterbury, New Zealand. May 22nd, 2015 (oral presentation).

Uster, B., Trumm, D., Pope, J., Weber P., O'Sullivan, A.D., Weisener, C., Diloreto, Z.A. (2014) *Waste Mussel Shells to Treat Acid Mine Drainage: A New Zealand Initiative.* Reclamation Matters (official publication of the American Society of Mining and Reclamation), Fall 2014. pp. 23-27.

(http://www.asmr.us/Publications/ReclamationMatters/2014/Fall2014Final.pdf)

An amended version of this article will be republished in the Canadian Land Reclamation Association Journal (Fall/Winter 2015, Vol. 15, Issue 2).

CHAPTER 1

GENERAL INTRODUCTION, RESEARCH OBJECTIVES & THESIS LAYOUT

1.1 Introduction

Mining activities produce a large amount of waste materials. These materials, known as waste-rock (i.e. the overburden which does not contain metals in economically profitable concentrations) or tailings (i.e. the remaining sludge after the ore has been processed and the valuable elements have been extracted) are usually deposited in constructed impoundments near the mine sites. These wastes usually contain sulfide minerals (e.g. pyrite, pyrrhotite, chalcopyrite, etc.) which will react with meteoric water and atmospheric oxygen and result in the generation of so-called mining-influenced water (MIW). MIW can be strongly acidic (pH between 2 and 4 in most cases), contain high concentrations of metals and metalloids (Fe, Al, Mn, Zn, Ni, Cu, As, Se, etc.), and sulfates (SO_4^{2-}). Mining activities, by increasing the sulfide minerals accessibility and their surface area, dramatically increase the generation rates of MIW.

MIW, and especially its acidic subsets commonly referred as acid mine drainage (AMD) or acid rock drainage (ARD), creates serious environmental challenges worldwide, and is regarded as the most serious environmental problem related to mining activities (Kalin 2004; Neculita 2010). The negative environmental impacts of MIW on neighboring aquatic systems including subsurface environments can be very detrimental. It has been reported that MIW

severely impacted about 20,000 km of streams and rivers in the Eastern United States (Ziemkeivic et al. 2003; Brown et al. 2002). In Europe, an estimated 5,000 km of watercourses were negatively impacted by MIW (Younger 2004). In 2003, the New Zealand West Coast Regional Council estimated that at least 125 km of streams were adversely affected by MIW in the North and Central areas of the West Coast of the South Island of New Zealand only (James 2003).

In addition, mine wastes and especially MIW, can have severe impacts on human health. Numerous examples of worldwide environmental and human damages can be found in the literature. For examples, researchers studied the impact of mining activities in Spain (Ruiz et al. 2008); Bolivia (Farag et al. 2015; Van Damme et al. 2008), Chile (Dold 2006), USA (Hendryx et al. 2012), China (Zhou et al. 2007) and New Zealand (Niyogi et al. 2013; Hogsden and Harding 2011; Harding 2005). Due to a legacy of unmitigated mining activities (e.g. thousands of abandoned mines produce MIW worldwide), and because MIW is a long-term problem (i.e. it is a self-perpetuating process, see Chapter 2 section 2.1), it carries an enormous financial burden for the affected governments and populations. For example, in the State of Pennsylvania only, it is was estimated that the cost of remediation of all the waterways impacted by MIW in 1997 would be US$ 15 billion (Rossman et al. 1997).

Therefore, the development of effective, sustainable and affordable treatment technologies to treat MIW is a crucial challenge for both the mining industry and the concerned governments. Multiple remediation strategies have been developed and successfully applied worldwide. Because conventional “active” treatment technologies such as lime-dosing are chemical and

energy-intensive, alternative cost-effective and environmentally-sensitive remediation strategies (so-called "passive" treatment technologies) have been developed over the past three to four decades. These "passive" technologies include a range of engineered wetlands and bioreactors relying on biogeochemical processes to mitigate acidity and immobilize contaminants (i.e. metals, salts) from MIW. Although the designs of such systems and their short-term efficiencies are relatively well quantified, their long-term effectiveness is still poorly understood due to the inherent complex biogeochemical processes on which MIW passive treatment relies.

1.2 Research Objectives and Hypothesis

Previous research has demonstrated that sulfate-reducing bioreactors (SRBR) using waste materials are very efficient in treating MIW (Neculita 2010). Dozens of organic waste materials have already been tested and more than ninety publications dealing with the use of organic mixtures have been identified. However, few studies have looked at using alternative alkaline material instead of the commonly used limestone. One previous research study, conducted at the University of Canterbury, found excellent contaminant removal efficiencies in SRBR treating MIW from active New Zealand coal mines using raw waste mussel shells as a source of alkalinity (McCauley et al. 2009; McCauley 2011). These waste mussel shells, a large volumetric waste produced nationally, are currently disposed of in landfills and alternative re-use options are needed. Although the general bioreactor design criteria have been established, there is potential to improve on performance with new substrate materials. In addition, a strict and methodically sound comparison between mussel shell and limestone has not been done, and the exact biogeochemical processes responsible for passive treatment,

especially the metal removal mechanisms, are still poorly understood, thus providing niche research questions that should be investigated.

The overall goal of the present research was to investigate some of the critical biogeochemical processes occurring in lab-scale SRBR (i.e. mesocosms) treating MIW and using mussel shells in the reactive mixture. For comparative purposes, a second reactive mixture containing limestone instead of mussel shells was also tested. In addition, two hydraulic retention times (HRT), which is a critical design parameter, were also investigated. Data attained from laboratory experiments were used to assess the efficiencies of the different treatment systems tested (mussel shell vs. limestone; short vs. long HRT), and to elucidate the fate of contaminants under the different operational conditions. Autopsies of the reactors' spent substrates, including chemical and mineralogical analyses were conducted to study the metal removal mechanisms.

The main hypotheses of this research were that the treatment efficacy is influenced by (1) the alkaline material, and (2) by the HRT. Based on the fact that mussel shells have a different and potentially more dissolvable calcium carbonate composition compared to limestone, it was anticipated that the mixture containing mussel shells would result in a better contaminant removal compared to the mixture containing limestone. Similarly, it was expected that the longer HRT affording greater contact time between the MIW and the substrate materials would also result in a better the treatment efficacy.

The main research questions associated with this research were:

- How does MIW treatment efficiency (in terms of acidity mitigation, metal and sulfate removal) change as a function of different alkalinity sources (waste mussel shell vs. limestone)?
- How does MIW treatment efficiency (in terms of acidity mitigation, metal and sulfate removal) change as a function of different HRT (short HRT of ≤ 3 days vs. long HRT of ≥ 9 days)?
- In which forms are the metals retained in the substrates mixtures (what are the metal removal mechanisms taking place)?

1.3 Thesis layout

Chapter 2 *(Literature review)*: The literature review first introduces the general geochemistry associated with the generation of MIW. Then the main processes associated with the treatment of MIW in SRBR are presented.

Chapter 3 (*Substrate materials characteristics and experimental design*): This chapter describes the substrate materials to be used in the SRBR and discusses the analyses performed on these materials prior to their use in the reactors. The results include geotechnical, chemical and mineralogical data. In addition, the experimental design of the treatability study is presented.

Chapter 4 (*Mine-influenced water treatability study*): This chapter presents the results of a 10-month flow-through treatability study (two 5-month treatment periods separated by a 2-month resting period). This study included numerous water geochemistry analyses performed on the influent, pore-water and effluent samples. Results from the four SRBR systems tested in this study (two hydraulic retention times and two alkaline materials) are discussed and compared with each other and with data from other similar studies.

Chapter 5 (*Post-treatment substrate autopsies: Investigating metals removal processes*): This chapter presents the post-treatment analyses performed on the spent substrates used in the SRBR. Geochemical and mineralogical analyses, including a sequential extraction experiment, are used to comprehend the metal removal processes, and the results from a separate adsorption study are presented. The model PHREEQC is used in comparison with the sequential extraction results to better understand the processes associated with the metal removal mechanisms.

Chapter 6 (*General conclusions and future work recommendations*): This final chapter summarizes and critiques the findings from each of the individual chapters. In addition, recommendations and suggestions for future work are provided.

CHAPTER 2

LITERATURE REVIEW: MINING-INFLUENCED WATER & SULFATE-REDUCING SYSTEMS

2.1 Geochemistry of mining-influenced water

The generation of mining-influenced water (MIW) is a process during which sulfide minerals are oxidized by the presence of oxygen and water. The weathering of sulfide minerals (e.g. pyrite, pyrrhotite, chalcopyrite, etc.) occurs when ore deposits are exposed to atmospheric conditions. This weathering process can sometimes occur in pristine areas, but it is considerably promoted by mining activities. Through mine workings, the accessibility of air and water as well as the reactive surface area of the metal ores are greatly increased (i.e. the sulfide minerals previously kept underground in reducing and anaerobic conditions are exposed to oxidizing and aerobic conditions), resulting in greater rates of geochemical weathering in mined areas (Blowes et al. 2003; Dold 2003; Plumlee 1999).

MIW has three main components: (1) acidity, (2) metals, and (3) sulfates. Although MIW chemical signatures display a large variability, most MIW occurrences are strongly acidic (pH values in the range of 2 to 4), and contain high concentrations of dissolved metals (most commonly Fe, Al, Mn, Zn, Cu; total concentrations can be as high as several hundreds of mg per liter) and high concentrations of sulfates (up to several grams per liter). Table 2-1 shows a range of MIW chemical signatures from metal and coal mines in the USA and in New Zealand and a comparison with the average world river water quality.

Table 2-1. Chemical signatures of various MIW compared to average world water river water. Sulfate and metal concentrations are in mg/L.

	Iron Mountain, CA, USA[a]	Leviathan Mine, CA, USA[a]	Cameron Mine, PA, USA[a]	Bellevue Mine, NZ[b]	X Mine, NZ[c]	Average World River Water[a]
pH	- 2.5	1.85	4	3.01	2.69	6.5-8.5
SO_4^{2-}	760,000	11,200	510	750	1,636	5.3-16.8
Fe	111,000	2,510	49	98.4	27.4	0.0066
Al	1,420	623	5.4	43.2	15.5	0.032
Mn	23	9.32	6.1	0.92	15.8	0.034
Cu	4,760	9.64	0.0014	0.002	0.24	0.0015
Zn	23,500	2.62	0.36	0.37	4.8	0.0006
Ni	3.70	13.0	0.70	0.13	1.0	0.00015
Cd	211	0.34	0.038	n.d.	0.003	0.00008

Source of data: a = Nordstrom (2011b); b = Pope et al. (2010); c: this study; n.d. not determined.

MIW draining by gravity from active or abandoned mine sites into surrounding surface and subsurface water systems can last for a few decades to several centuries in the worst cases, and is therefore considered the most serious long-term environmental problem related with mining activities (Kalin 2004). The flow of MIW is also highly variable, ranging from a few cubic meters per day to a few cubic meters per second (Brunet 2000). MIW are highly detrimental to natural waters, killing enormous quantities of fish and benthic organisms, destroying crops and making many streams, rivers, and lakes unfit for most beneficial purposes. In some cases MIW can even compromise fresh water resources for domestic and industrial uses (Younger 2004).

Three components of acidity exist in MIW chemistry: (1) the proton acidity, which is a measure of the free proton ions (H^+) in solution (i.e. the pH); (2) the organic acidity, which is related to the dissolved organic compounds, and (3) the mineral acidity associated with the dissolved metals that will undergo hydrolysis resulting in more proton production. Ordinarily, the organic content in MIW is low and therefore the organic acidity is low too. Surprisingly the pH is neither the largest contributor (until it reaches a pH value of 3.5 or less), and the mineral acidy is usually the main component (Watzlaf et al. 2004).

Acidity is commonly referred to in calcium carbonate milli-equivalents (mg/L of $CaCO_3$ eq.) and can be calculated using Equation 2.1. This equation can be modified to include the acidity contribution of other metals such as copper, nickel, zinc, etc., if they are present in solution at a significant concentration (Kirby and Cravotta 2005).

$$Acidity_{calc.} = 50\ (2\ [Fe^{2+}]/55.85 + 3\ [Fe^{3+}]/55.85 + 3\ [Al^{3+}]/26.98 + 2\ [Mn^{2+}]/54.94 + 1000\ (10^{-pH})) \quad (2.1)$$

Where: [Metal] = metal concentrations (mg/L)

The counterpart of acidity is alkalinity. Total alkalinity in MIW is typically computed using Equation 2.2 (Kirby and Cravotta 2005), where each component is in mol/L.

$$\text{Total alkalinity}_{calc.} = [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+] \quad (2.2)$$

In mine water the principal source of alkalinity arises from the presence of dissolved carbonate minerals (HCO_3^- and CO_3^{2-}), but some alkalinity can also result from dissolved silicates, borate, phosphate, ammonia and organic ligands. It is important to keep in mind that alkalinity and acidity are not exclusive terms and that water can simultaneously contain both. Operationally, a MIW is defined as net acidic when acidity is higher than alkalinity or net alkaline when acidity is lower than alkalinity (Kirby and Cravotta 2005).

The main metals present in MIW are iron, aluminum, manganese, copper, zinc, cadmium, arsenic and nickel and many other trace metals can be found at various concentrations. When assessing the ecotoxicity of MIW, chemical speciation and synergetic effects of the metals must be taken into consideration because they will influence the overall toxicity as well as their respective bioavailability (Brunet 2000).

The biogeochemical reactions leading to the formation of MIW are numerous and quite complex. Many factors influence the MIW generation, the most important being the presence of water for dissolution and gravitational transportation, the initial pH and redox potential of the source water body, the oxygen diffusion rate, the microbial activity, and the presence of neutralizing minerals (e.g. carbonates, silicates). Over the long-term, climate (i.e. precipitation, temperature and temperature variations) also has an influence on MIW generation (Nordstrom 2009, 2011a).

Equations 2.3 to 2.10 describe the generation of MIW through the oxidation of pyrite (the most common sulfide mineral). The very first step is the oxidation of pyrite by molecular oxygen (Eq. 2.3). This reaction will release protons and consequently acidify the environment. Note that the oxidation of 1 mole of pyrite releases 2 moles of protons.

$$FeS_2(s) + 3.5\ O_2(g) + H_2O(l) \rightarrow Fe^{2+}(aq) + 2\ SO_4^{2-}(aq) + 2\ H^+(aq) \quad (2.3)$$

Pyrite + oxygen + water → ferrous iron + sulfate + proton

Where: (s) = solids; (g) = gas; (l) = liquid; (aq) = aqueous (dissolved).

Pyrite can also be oxidized by ferric iron (Fe^{3+}) (Eq. 2.4). This reaction is 2 to 3 orders of magnitude faster than the oxidation of pyrite by oxygen (Eq. 2.3), and release 16 moles of protons per mole of pyrite.

$$FeS_2(s) + 14\ Fe^{3+}(aq) + 8\ H_2O(l) \rightarrow 15\ Fe^{2+}(aq) + 2\ SO_4^{2-}(aq) + 16\ H^+(aq) \quad (2.4)$$

Pyrite + ferric iron + water → ferrous iron + sulfate + proton

In order to have ferric iron in solution, the pH must be lower than 4 otherwise Fe will undergo a hydrolysis reaction which will produce an amorphous iron hydroxide and release more protons in solution (Eq. 2.5).

$$Fe^{3+}(aq) + 3\ H_2O(l) \rightarrow Fe(OH)_3(s) + 3\ H^+(aq) \quad (2.5)$$

Ferric iron + water → iron hydroxide + proton

Hence, the generation of ferric iron via the oxidation of ferrous iron (Eq. 2.6) is a crucial step in MIW geochemistry and is considered to be the rate determining step of MIW generation (Nordstrom and Alpers 1999a).

$$Fe^{2+}(aq) + 0.25\ O_2(g) + H^+(aq) \rightarrow Fe^{3+}(aq) + 0.5\ H_2O(l) \quad (2.6)$$

Ferrous iron + oxygen + proton → ferric iron + water

The kinetic of Eq. 2.6 is influenced by numerous factors including the oxygen concentration and diffusion rate, the sulfate concentration, the presence of light, and the pH. Additionally, the oxidation of ferrous iron (Eq. 2.6) is both an abiotic and a biotic process, and these two mechanisms are pH dependent. The abiotic reaction is very fast when pH is above 8 (occur in seconds) but extremely slow when pH is lower than 5 (occurs in days). In contrast, the biologically driven oxidation of ferrous iron is maximal when pH is low (pH 2-3), but almost inexistent if the pH is above 5 (Mills 1999; Younger et al. 2002).

Eq. 2.6 is crucial because it allows MIW generation to be self-perpetuating. For example, ferrous iron produced by Eq. 2.3 and 2.4 will undergo oxidation and produce ferric iron (Eq. 2.6), which will then oxidize more pyrite and subsequently more ferrous iron (Eq. 2.4).

Consequently MIW generation is a self-sustaining process that will last until all the pyrite and/or all the ferric iron is consumed. For example, Nordstrom and Alpers (1999b), estimated that the Iron Mountain Superfund in California could potentially generate severe MIW for up to 3,000 years if no preventive measures were taken.

An overall summarizing reaction can be written as the following (Eq. 2.7). This reaction produces 4 moles of proton.

$$FeS_2(s) + 3.75\ O_2(g) + 3.5\ H_2O(l) \rightarrow Fe(OH)_3(s) + 2\ SO_4^{2-}(aq) + 4\ H^+(aq) \qquad (2.7)$$

Pyrite + oxygen + water → iron hydroxide + sulfates + proton

As aluminum minerals are ubiquitous in soils and riverbed sediments and usually associated with coal deposits, Al is (after Fe) the second most important metal found in MIW. In natural aqueous systems Al has only one oxidation state (i.e. Al^{3+}) and its presence is only governed by the pH, which influences the solubility of an amorphous aluminum hydroxide ($Al(OH)_3$) (Stumm and Morgan 1996). Changes in the redox potential have no effect on aluminum concentration as long as the pH does not change simultaneously. At low pH, the aluminum concentration is high because the solubility of $Al(OH)_3$ is high, but if the pH is higher than approximately 4.5 the concentration of aluminum is likely to be very low (usually less than 1 mg/L) (Younger et al. 2002).

Aluminum in MIW primarily comes from the dissolution of alumino-silicate clay minerals such as microcline ($KAlSi_3O_8$), kaolinite ($Al_2Si_2O_5(OH)_4$) and muscovite ($KAl_2(Al,Si)_3O_{10}(OH,F)_2$) (Dold 2003). For example, the dissolution of microcline will produce silicic acid and kaolinite as shown in Eq. 2.8. Kaolinite will be further dissolved to release trivalent aluminum cations (Eq. 2.9). Then, depending of the pH, aluminum cations can undergo hydrolysis resulting in the precipitation of an aluminum hydroxide and in the release of more proton acidity (Eq. 2.10). The hydrolysis reaction will occur if the pH is equal or greater than 4.5 (Younger et al. 2002; Stumm and Morgan 1996).

$$KAlSi_3O_8(s) + 2\ H^+(aq) + 9\ H_2O(l) \rightarrow 4\ H_4SiO_4(aq) + Al_2Si_2O_5(OH)_4(s) + 2\ K^+(aq) \qquad (2.8)$$

Microcline + proton + water → silicic acid + kaolinite + potassium

$$Al_2Si_2O_5(OH)_4(s) + 6\ H^+(aq) \rightarrow 2\ Al^{3+}(aq) + 2\ H_4SiO_4(aq) + H_2O(l) \qquad (2.9)$$

Kaolinite + proton + water → aluminum cation + silicic acid + water

$$2\ Al^{3+}(aq) + 3\ H_2O(l) \rightarrow Al(OH)_3(s) + 3\ H^+(aq) \qquad (2.10)$$

Aluminum cation + water → aluminum hydroxide + proton

The chemical reactions shown above summarize the basic geochemistry of MIW generation, but in reality many more reactions may occur and the influence of microorganisms render the overall process of sulfide mineral dissolution much more difficult to assess. Indeed

microorganisms are ubiquitous in mining environments, and through their metabolism they affect metal mobilization, retardation and transformation (Ledin and Pedersen 1996; Mills 1999). An extensive body of literature exists on MIW generation and MIW geochemistry, and interested readers can refer to the following studies and reviews: Bigham and Nordstrom (2000), Blowes (2003), Brunet (2000), Dold (2003), Ehrlich and Newman (2008b), Nordstrom and Alpers (1999a), Nordstrom et al. (2015), Plumlee (1999), Younger et al. (2002), Zobrist and Giger (2013). In addition, information on the MIW geochemistry specific to the New Zealand geologic and climatic context can be found in the following studies: Campbell et al. (2001), Cavanagh et al. (2010), Davies et al. (2011), Pope et al. (2010), Trumm (2007).

2.2 Treatment of mining-influenced water

Numerous methods, either based on continuously applying chemicals (i.e. active treatment) or relying on biogeochemical processes within a low maintenance system (i.e. passive treatment), have been developed to treat MIW. Each technique presents advantages and drawbacks and the final treatment system choice depends on various parameters such as MIW geochemical signature and flow quantity, access to the site, available land area for the treatment system, capital and maintenance costs, legislation, etc. (Johnson and Hallberg 2005a; Skousen et al. 2000).

2.2.1 Active systems

Active treatment entails chemically treating MIW in an engineered water treatment plant where various chemicals are applied to remove acidity and dissolved metals. These

conventional treatment techniques generally work well and are a reliable technology, but they are very expensive (for both capital and maintenance costs) in terms of energy (e.g. electrical power to pump, mix, heat and aerate the tanks) and chemicals. Because active treatments allow a more precise and rapid control, they are more suited to the management of MIW during active mine operations and for treating large volumes of MIW. On average, active treatment using lime dosing costs about $NZ 324 per tonne of acidity removed (pers. comm. Paul Weber, former Environmental Manager, Solid Energy, New Zealand).

The most common alkalinity-generating materials for lime dosing are calcium oxide (CaO) or calcium hydroxide ($Ca(OH)_2$). Alternatively, other chemicals can be used such as sodium hydroxide (NaOH), sodium carbonate ($NaCO_3$), magnesium oxide (MgO), magnesium hydroxide (MgOH) and many others depending on the choice of active treatment and the required quality of the effluent water. The interested reader can refer to the following references for extensive details on active treatment of MIW: Brown et al. (2002), Skousen et al. (1998; 2000), Trumm (2010), Younger et al. (2002).

2.2.2 Passive systems

Passive MIW treatments originated from the observation that natural systems such as wetlands can improve water quality (Wieder and Lang 1982, 1984). Therefore, constructed ecosystems can afford the engineer an opportunity to capitalize on naturally occurring biogeochemical processes employing non-synthetic media. Furthermore, passive treatment systems do not require as much (if any) fossil energy inputs since systems can be integrated into the local topography utilizing natural gradient for flow conveyance. And since MIW is a self-renewing

process that can last for centuries, the treatment should be designed as a long term solution (Kalin et al. 2006). The term "passive" treatment can sometimes be misleading because most of those systems are not maintenance free, but typically incur lower maintenance than active treatment. The following definition, found in the forewords of the document PIRAMID Design Guidelines (PIRAMID 2003) is one of the most comprehensive:

> *"Passive treatment is the deliberate improvement of water quality using only naturally-available energy sources (e.g. gravity, microbial metabolic energy, photosynthesis), in systems which require only infrequent (albeit regular) maintenance in order to operate effectively over the entire system design life."*

The principal advantages of passive treatment compared to active treatment include: low maintenance requirements, relatively small operating and capital costs, use of non-hazardous materials, systems operating for relatively long periods of time unattended, and a better integration into the surrounding environment sometimes even providing natural habitat (O'Sullivan 2005). In some cases the use of passive treatment even embraces cultural and educational values. However, larger land footprints are required for passive treatment and they are not receptive to shock-loading (i.e. excessive contaminant loading rates). Numerous types of passive treatment systems have been developed and the choice of a particular technology over another depends on factors such as the chemical signature of the MIW (e.g. Fe and Al concentrations, dissolved oxygen concentration, flow) and the available land area (Gusek and Wildeman 2002a; Trumm 2010; Wildeman et al. 2006; Younger et al. 2002).

Sulfate-reducing bioreactor is a promising type of passive treatment that has gained prevalence in recent years due to its success at concurrently removing the main contaminants of acidity, metals and sulfates from MIW, and will be discussed in detail in the following sections. Other passive treatment technologies, outside the scope of the present research, are extensively documented in the literature (Gusek 2008; Hedin et al. 1994; Higgins et al. 2003; O'Sullivan 2005; PIRAMID 2003; Rose 2010; Skousen et al. 1998; Skousen et al. 2000; Watzlaf et al. 2004; Wildeman et al. 2006; Younger et al. 2002; Ziemkeivic et al. 2003).

2.2.3 Sulfate-reducing bioreactors

A SRBR relies on the microbial principle of sulfidogenesis to generate alkalinity and to remove metals and sulfates from solution (Chang et al. 2000; ITRC 2012; Sheoran et al. 2010). The microorganisms responsible for this process are called sulfate-reducing bacteria (SRB). SRB species are found in diverse environmental locations like geothermal vents, wetland sediments, and in most ruminant animal digestive systems.

During sulfidogenesis, SRB reduce sulfates (SO_4^{2-}) to sulfides (H_2S, HS^-) via a dissimilatory bioenergetic metabolism, coupling the oxidation of an electron donor (i.e. provided by the organic carbon substrate) to the reduction of a terminal electron acceptor (i.e. the sulfates). The energy gained in this way is used by SRB for growth and development as they metabolize. Equations 2.11 and 2.12 show the basic oxidation of a simple carbon source (represented as CH_2O), the reduction of sulfates to sulfides and the concomitant generation of

bicarbonates, respectively (Postgate 1979; Tang et al. 2009). The sulfides produced through sulfidogenesis will then react with divalent metals present in the MIW to form insoluble metal sulfides (see Equation 2.20 and section on metal removal below). In these bioreactors, SRB activity is confirmed by lower redox potential values, the presence of free sulfides, and a smaller concentration of sulfates in the effluent than in the influent (Johnson and Hallberg 2005b).

$$2\ CH_2O(s) + SO_4^{2-}(aq) \rightarrow S^{2-}(aq) + 2\ CO_2(aq) + 2\ H_2O(l) \quad (2.11)$$

Simple organic source + sulfate → sulfide + carbon dioxide + water

$$S^{2-}(aq) + 2\ CO_2(aq) + 2\ H_2O(l) \rightarrow 2\ HCO_3^-(aq) + H_2S(g) \quad (2.12)$$

Sulfide + carbon dioxide + water → bicarbonate + hydrogen sulfide

SRB are heterotrophic (i.e. organisms that solely use organic carbon as a carbon source) or autotrophic (i.e. use inorganic carbon as a carbon source) and are considered to be strictly obligate anaerobes (i.e. anaerobes organisms are harmed by the presence of oxygen). Nevertheless, aerobic growth was found possible at the oxic/anoxic interface and in anoxic microenvironments within aerobic systems (Willow and Cohen 2003). SRB can use a number of simple carbon and electron sources such as low weight molecular compounds (e.g. methanol, ethanol or lactate); simple carbohydrates monomers (e.g. glucose) or polylactic acids (e.g. acetic acid, lactic acid, pyruvic acid), and hydrogen (Postgate 1979; Tang et al. 2009). Depending on the carbon source, the SRB will consume one to two moles of proton and

produce approximately two moles of bicarbonate (HCO_3^-) per mole of sulfate reduced. The exact numbers depends on the electron donor as shown in Equations 2.13 to 2.16 illustrating the reduction of sulfate using different simple carbon sources.

$$CH_3COO^-(aq) + SO_4^{2-}(aq) + H^+(aq) \rightarrow H_2S(aq) + 2\ HCO_3^-(aq) \quad (2.13)$$

Acetate + sulfate + proton → hydrogen sulfide + bicarbonate

$$CH_3CHOHCOO^-(aq) + 3/2\ SO_4^{2-}(aq) + H^+(aq) \rightarrow 3/2\ H_2S(aq) + 3\ HCO_3^-(aq) \quad (2.14)$$

Lactate + sulfate + proton → hydrogen sulfide + bicarbonate

$$4\ CH_3CH_2COO^-(aq) + 7\ SO_4^{2-}(aq) + 6\ H^+(aq) \rightarrow 7\ H_2S(aq) + 12\ HCO_3^-(aq) \quad (2.15)$$

Propionate + sulfate + proton → hydrogen sulfide + bicarbonate

$$4\ H_2(aq) + SO_4^{2-}(aq) + 2\ H^+(aq) \rightarrow H_2S(aq) + 4\ H_2O(l) \quad (2.16)$$

Hydrogen + sulfate + proton → hydrogen sulfide + water

In the case of SRBR using complex organic wastes as electron donor, other microorganisms like fermenting and cellulolytic bacteria are necessary to the proper functioning of SRBR (e.g. these microorganisms are responsible for the biodegradation of the substrate and therefore for the supply of simple organic carbon sources to the SRB). General microbial processes occurring in SRBR's complex substrates are discussed in more details in the following section.

In addition to a simple carbon source, SRB require optimal conditions to thrive and various parameters are of paramount importance for an optimal treatment efficacy, and should therefore be carefully monitored. Of those, the pH is certainly the most crucial because it will influence the solubility of the metals, the biodegradability of the substrate, and the activity of the microorganisms. Although some strains of acidophilic SRB have been isolated from various environments with low pH (pH < 3), a pH above 5.5 is recommended for an efficient treatment process (pH < 5 inhibits the reduction of sulfates and increases the solubility of metal sulfides) (Dvorak et al. 1992; Willow and Cohen 2003; Zagury et al. 2005). In addition, mixed SRB cultures have been reported to be more tolerant to strongly acidic conditions compared to pure single strain cultures (Kolmert and Johnson 2001).

Even though sulfate reduction has been observed in passive treatment systems with a positive oxido-reduction potential (ORP), an anoxic and reduced microenvironment (ORP < -100mV) is recommended for an optimal activity of the SRB (Postgate 1979; Zagury et al. 2005). Because ORP measurements are often made at the outlet of the treatment systems, it is likely that the SRB survived in anoxic and reduced microenvironments within these oxic systems. In addition, several authors agree that the lower the redox potential is, the higher the SRB activity will be (Postgate 1979; Zagury et al. 2006). In SRBR, the reduction of the ORP is mainly attributed to the activity of the fermenting bacteria (ITRC 2012; Kadlec and Wallace 2009).

Temperature will also affect the overall treatment efficacy by influencing the kinetics of the organic substrate decomposition, the solubility of hydrogen sulfide, and the bacterial growth and activity (Sheoran et al. 2010; Zagury et al. 2005). Postgate (1979) states that even if SRB can tolerate temperatures between -5°C and 75°C, a low temperature will negatively influence their growth and development, and a temperature above 6°C is recommended. Because cellulose degraders and fermenting bacteria, responsible for the substrate decomposition (i.e. responsible for the continuous supply of simple organic carbon sources for the SRB), are more affected by low temperature than the SRB, some studies suggest that the overall rate of sulfate reduction might by reduced by 50 % if the temperature is less than 10°C compare to a temperature of more than 20°C (Sheoran et al. 2010). Others argue that the temperature will mostly affect the SRB acclimatization, but once established, the bacterial community will be less affected by low temperature and by temperature variations (Tsukamoto et al. 2004). Kuyucak et al. (2006) reported that winter freezing had little effect on a well establish SRB population activity. In addition, the temperature will affect the rate constants of the different chemical reactions, which will usually increase with an increasing temperature.

Surprisingly, sulfides and various sulfur compounds also can inhibit SRB activity, with increasing effect following the order of sulfates < thiosulfates < sulfites < total sulfides < hydrogen sulfides (Zagury et al. 2005). Reis et al. (1992) reported that un-dissociated hydrogen sulfide has a toxic but reversible effect on SRB (e.g. the neutral molecules being able to easily enter the cell membrane). In addition, while at low concentrations heavy metals can have a stimulatory influence on SRB activity, they can become inhibitory and even lethal to the microorganisms if their concentrations become too high (Utgikar et al. 2002).

2.2.3.1 Microbial and physical considerations of the substrates

One of the most critical factors in SRBR design is the supply of a suitable and continuously available organic source from which the bacteria will obtain energy and nutrients (Neculita and Zagury 2008). Because dissolved organic carbon concentration in MIW is typically lower than 10 mg/L, an additional appropriate organic carbon source is needed to promote SRB activity (Kolmert and Johnson 2001). A large number of organic substrates, consisting either of simple or complex organic substances have been shown to be efficient in SRBR (Table 2-2).

Table 2-2. Some organic materials used in SRBR substrates.

Substrate category	Specific substrate materials
Simple organic liquids	methanol, ethanol, acetate, lactate, glucose
Compost	spent mushrooms, municipal, leaves, conifer
Manure	cow, poultry, cattle, sheep, goat, buffalo, horse
Plant products	alfalfa, straw, hay, leaves, walnut shells, peat moss
Wood products	woodchips, sawdust, bark
Sludges	sewage, digested, paper pulp
Other	fly ash, molasses, cheese whey

The actual choice is usually made on the availability and the cost of the added electron donor per unit of reduced sulfate; taking into account that the remaining contaminants in the treated effluent must be as low as possible and/or easy to remove with another treatment system (Zagury et al. 2005). Yet, when used in large scale SRBR, simple organic compounds should not be used because of their expensive prices. Therefore, cheap complex organic sources such as waste materials from agricultural, forest, and food industry should be employed. In addition

to carbon, other nutrients including nitrogen and phosphate are required for the microorganisms to thrive (Ehrlich and Newman 2008a). Sulfate and metal concentrations in the materials should also be analyzed prior to their use in passive systems in order to avoid the use of a material that contains high contaminant concentrations which could be detrimental to the overall treatment performance (e.g. wood wastes can contain high concentrations of chromium or arsenic if the wood was treated with chemical fire retardants; municipal composts can contain high concentration of copper and lead from urban activities).

Due to the complex biogeochemical interactions occurring in SRBR substrates there is no consensus on the best materials or mixtures to use, but several studies have found that the removal processes were more effective when a combination of easily biodegradable substances and more recalcitrant ones were used (Gibert et al. 2004; Neculita and Zagury 2008). In addition, fresh materials are usually preferred to older substances as they have not been weathered and therefore still contain high amount of readily available simple carbon substances. A certain number of indicators can be used in order to better select the different organic sources. Zagury et al. (2006) and Gibert et al. (2004) indicate that the following criteria should be investigated: total organic carbon content; total nitrogen content; carbon/nitrogen ratio; cellulose and hemicellulose content; lignin content; cellulose/lignin ratio and easily available substances content (EAS).

Although SRB are the species driving the desired biogeochemical treatment processes in SRBR (i.e. the reduction of sulfate to sulfide), a large and complex microbial ecosystem is required to ensure the performance and the longevity of these passive treatment systems

(Logan et al. 2003). As SRB cannot directly use complex organic substrates, a complete microbial community is required to sequentially degrade the complex organic carbon sources into utilizable substances. In other words, a sequential bacterial community is essential as each group of bacteria provide nutrients for the next group.

The most important groups are: (1) the cellulose degraders (cellulolytic bacteria), (2) the fermenting bacteria (fermenters), and (3) the SRB (sulfate reducers). The first group hydrolyzes the organic substrate, thus breaking down the large molecular weight compounds into lower molecular weight compounds like glucose. Then, the fermenting bacteria use the newly released low molecular weight compounds and convert them into simpler molecules which are readily utilizable by the SRB.

As an example, equations 2.17 and 2.18 show the sequential breakdown of cellulose into carbon dioxide and ethanol.

$(C_6H_{10}O_5)n + n\ H_2O(l) \rightarrow n\ C_6H_{12}O_6$ (2.17)

Cellulose + water → glucose

$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH(aq) + 2\ CO_2(g)$ (2.18)

Glucose → ethanol + carbon dioxide

Figure 2-1 shows some of the multiple microbial pathways involved in the decomposition of the organic material in complex SRBR substrates.

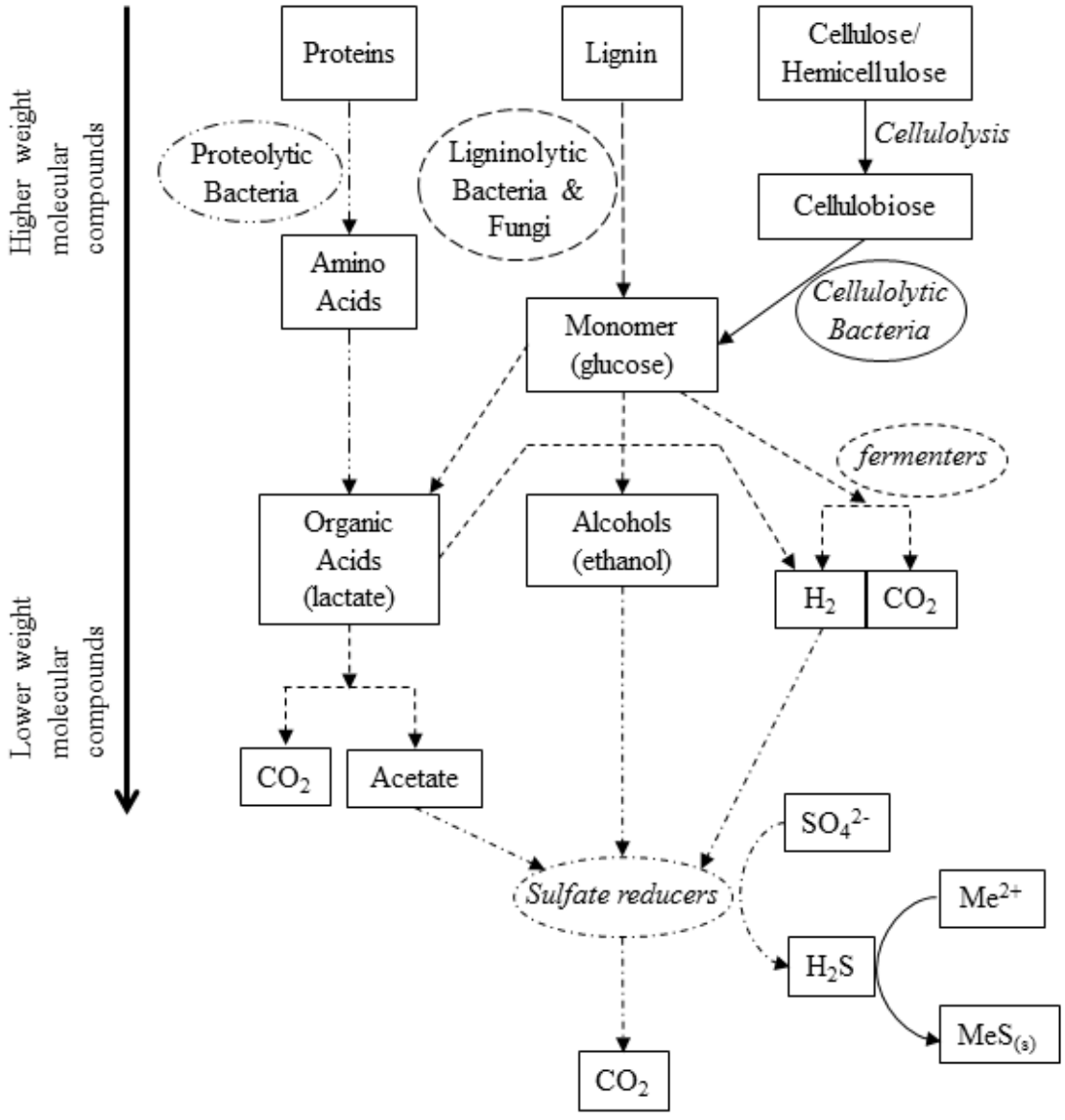


Figure 2-1. Anaerobic microbial pathways of organic substrate digestion (modified after Logan et al. (2005).

In a SRBR using a mixture of complex organic materials, the SRB will first consume the simple low molecular weight compounds. Then, when all simple organic substances are depleted, the rate of sulfate reduction will primarily depend on the rate of cellulose breakdown, and many authors consider that, when all simple organic compounds have been consumed, the cellulose degradation is the rate-limiting step for long-term performance of SRBR (Ledin and Pedersen 1996; Logan et al. 2003; Zagury et al. 2006). Ultimately, the rate and extent of substrate utilization will control the performance and the longevity of a SRBR. For this reason, cellulose degraders and fermenting bacteria are fundamental microorganisms in SRBR, and the most important part of the overall bacterial population consists of those two groups of bacteria. While SRB usually count only for 1 to 2 % of the total microbial community, it is important to note that the abundance of SRB have never been found to be a limiting factor in the overall performance of SRBR (Pruden et al. 2006). Lignin is a major chemical component of all complex organic substrate derived from wood and plant's cell walls. In opposition with cellulose and hemicelluloses, it is hardly biodegraded by microorganisms. Gibert et al. (2004) demonstrated that there is a negative relationship between the lignin content and the efficacy of the organic material to decompose and therefore to sustain the microbial activity.

In SRBR using a complex reactive mixture substrate, it is recommended to allow a certain period of time for the system to mature (i.e. the acclimatization period), in other words, to give sufficient time for the microorganisms to acclimate and to develop an adequate and large complex microbial population capable of sustaining the degradation of the complex organic substrate and the reduction of sulfate (Neculita et al. 2007a).

Regardless of its biodegradability, the physical characteristics of the solid substrate also have a significant influence on the reactor performance as SRB and other microorganisms involved in the treatment need a solid surface to attach to and to establish favorable reduced microenvironments. In addition, the hydraulic conductivity (which is related to the permeability), on which depends the hydraulic retention time (HRT; i.e. the contact time between the MIW and the substrate) is of primary importance (Benner et al. 2002). According to Younger et al. (2002), the HRT, which is specific to each reactor, is one of the most important operational parameters, yet one of the most difficult to optimize and achieve. For example, if the HRT is too short, the bacteria will not have enough time to reduce the sulfates and to precipitate the metals. On the other hand, if the HRT is too long, the depletion of organic matter will occur rapidly and stop the treatment process. Microorganisms, via the accumulation of biomass and by-products, will induce physical changes in the substrate, thus potentially affecting the HRT over time. They will increase the density and tortuosity of the substrate, hence leading to pore-clogging, or could consume organics to the point where porosity increases, leading to more flow and the potential development of preferential channels. Overall, a large surface area (which is beneficial for sorption processes and bacterial attachment) needs to be balanced with large pore spaces and large void space to reduce risk of plugging and clogging (Lyew and J.D. 1997; Neculita et al. 2008a). For long-term performance of SRBR, the biodegradability of the substrate and the HRT are thought to be the most important factors, and problems like compaction, plugging and clogging, preferential flow formation, and exhaustion of carbon sources are the most frequent reported reasons of failure (Gusek 2004, 2005).

2.2.3.2 Alkalinity generation

In order to create a favorable environment for the SRB, alkalinity generation is required to mitigate the proton and mineral acidity produced during the formation of MIW. In reducing systems, the alkalinity is generated via two mechanisms. Some bicarbonate is produced through the microbial reduction of sulfates (Eq. 2.12). However, the alkalinity generated via the reduction reaction is usually not sufficient to neutralize the acidity, and most of the time an alkaline material must be added to the organic substrate mixture in order to increase the alkalinity generation (Thomas and Romanek 2002a; Watzlaf et al. 2004; Waybrant et al. 1998; Wildeman et al. 2006). Limestone, which consists mostly of carbonate calcium ($CaCO_3$), is the most common alkaline material used in MIW passive treatment because it is relatively cheap and frequently found in relative abundance near mining sites. Limestone dissolution, which is affected by the calcium carbonate content (i.e. the purity of the limestone), and by its reactive surface area, is described by equation 2.19.

$$CaCO_3(s) + H_2O\,(l) + CO_2 \rightarrow Ca^{2+}(aq) + 2\,HCO_3^-(aq) \qquad (2.19)$$

Calcium carbonate + proton → calcium + bicarbonate

A purity of ≥ 90 % $CaCO_3$ is usually recommended (US-EPA 2000; Watzlaf et al. 2004). In addition, the dissolution of $CaCO_3$ is faster in anoxic environments because of the higher partial pressure of dissolved CO_2 (e.g. due to the closed environment and the dissolution of organic matter) and because of the lesser formation of ferric and aluminum (oxy)hydroxides potentially coating the limestone, thus inhibiting its dissolution (Watzlaf et al. 2004).

The amount of limestone added in the reactive mixture varies between the studies, but 30 % of the total volume is usually recommended (Wildeman et al. 2006). Other alkaline materials such as bivalve shells, seafood wastes, chitin, fly ashes, and steel slag have been successfully used (Daubert and Brennan 2007; Goetz and Riefler 2014; Newcombe and Brennan 2009; Robinson-Lora and Brennan 2009; Simmons et al. 2001; Venot et al. 2008; Younger et al. 2002; Zagury et al. 1997).

2.2.3.3 Metal removal mechanisms

The mechanisms of metal removal in a bioreactor are numerous and vary over the life-span of the system (Kaksonen and Puhakka 2007; Neculita et al. 2008b). In addition, these mechanisms differ between metals and depend on the MIW geochemical signature (i.e. metal speciation and loading, pH) and the biogeochemical condition within the reactor (dissolved oxygen concentration, oxido-reduction potential, etc.). The main metal removal mechanisms are: (1) sorption, which includes adsorption and biosorption, (2) precipitation, mainly in the form of sulfides, but hydroxide, (oxy)hydroxide, oxide, and carbonate formation can also occur in oxic and/or highly alkaline microenvironments, and (3) filtration and sedimentation to retain the newly formed precipitates (Kaksonen and Puhakka 2007; Sheoran et al. 2010). Insoluble metal precipitates can reduce the substrate biodegradability (i.e. by coating the substrate materials, the metal precipitates reduce their accessibility).

Adsorption, which transfer the metallic ions from the solution to a solid phase, occurs on organic media, but also on iron, manganese and aluminum (oxy)hydroxides and oxides, and to a lesser degree on metal sulfides. It varies with the pH, and roughly increases linearly with it.

The rate and the magnitude of metal adsorption onto abiotic and biotic minerals generally increase with the sorbent abundance and crystallinity (Jong and Parry 2004a; Webster et al. 1998) and varies with the nature of the sorbent which itself varies with the pH (e.g. at pH ≈ 4 more Fe-(oxy)hydroxides are present compared to pH ≈ 5 where more Al-(oxy)hydroxides are present). In addition, the exact mineralogical composition and structure of the (oxy)hydroxides will influence the total adsorption capacity. Adsorption onto biogenic sulfide minerals has been less documented, but Jong and Parry (2004a) and Pósfai and Dunin-Borkowski (2006) found that the extent of adsorption increases with the sorbent abundance and the pH, but decreases with the initial metal concentration. Adsorption onto organic media will takes place until all sorption sites are saturated, and it is therefore an important, but finite, sequestration process upon start-up of a new system (Machemer and Wildeman 1992). Again, it usually occurs preferentially at higher pH because of less competition with protons (i.e. the sorbent surfaces are deprotonated at high pH resulting in enhanced metal binding capacity).

Biosorption, which is the adsorption of metals onto microorganisms and biofilms, is either a metabolic dependent (i.e. internal compartmentalization and extra-cellular precipitation by metabolites) or a metabolic independent mechanism (i.e. chemical adsorption onto the negatively charged cell walls). The processes depend on many factors like age and physical state of bacteria, nutrient availability during growth, presence of competitive ions and physico-chemical parameters like pH, ORP and temperature (Utgikar et al. 2000).

Precipitation of sulfides is the desired removal mechanism because metal sulfides are highly insoluble and less bio-available (Sheoran et al. (2010); Table 2-3, Figure 2-2). The hydrogen

sulfide produced during equation 2.12 reacts with the dissolved divalent metals (M^{2+}) to form insoluble metal sulfides, basically reversing the reactions occurring during MIW generation (Eq. 2.20; Stumm and Morgan (1996). In addition, the precipitation of metal sulfides does not cause a parallel increase in proton acidity (as the precipitation of hydroxides do), because the dissociation of H_2S is neutralized by an equal release of HCO_3^- during sulfate reduction (Eq. 2.12). Finally, metal sulfide sludge is generally more dense and stable than metal hydroxide sludge, and shows better dewatering and thickening properties (Huisman et al. 2006).

$$M^{2+}(aq) + H_2S(g) \rightarrow MS(s) + 2\ H^+(aq) \qquad (2.20)$$

Divalent metal + hydrogen sulfide → metal sulfide + proton

The precipitation of metal sulfide depends on the pH, the concentration of the dissolved metals and hydrogen sulfide, and the equilibrium constants for metal sulfide solubility. Sulfide precipitation is thought to become the predominant mechanism once sulfate-reduction conditions are established (Machemer and Wildeman 1992). According to their solubility products, the first sulfide minerals to precipitate as the pH increases are CuS followed by PbS, CdS, ZnS, NiS and FeS (Table 2-3). Despite, the fact that pyrite (FeS_2) is thermodynamically the most stable iron sulfide, several metastable iron sulfides are likely to precipitate. These include greigite (Fe_3S_4), mackinavite (FeS) and divers amorphous iron sulfides (Machemer and Wildeman 1992; Machemer et al. 1993).

In oxic microenvironments, where the dissolved oxygen concentration is higher, metal hydroxides, (oxy)hydroxides and oxides can also precipitate as the pH increases. This is especially the case for Fe, Al and Mn. In MIW where ferric iron is present, it can hydrolyze to form various (oxy)hydroxides, commonly referred as iron ochres (because of the red, orange or yellowish color of the iron salts). The exact composition of these salts varies with the pH. If the pH is high (pH > 8), ferrihydrite ($Fe(OH)_3$) will precipitate, but at circumneutral pH (6 to 8), various forms of amorphous iron hydroxides and goethite (FeOOH) are more common. When the pH is lower (pH < 5), sulfates can substitute for hydroxide groups (OH^-) and (oxy)hydroxysulfates, such as schwertmannite ($Fe_8O_8(OH)_6(SO_4)*nH_2O$) or jarosite ($KFe_3(SO_4)_2(OH)_6$), are likely to precipitate (Bigham et al. 1996; Blowes et al. 2003; Dold 2003).

As stated before, aluminum has only one oxidation state in aquatic environments (i.e. Al^{3+}) and its behavior is only governed by pH and is not affected by the redox conditions. Therefore its removal is mainly controlled by the solubility of amorphous aluminum hydroxides (e.g. $Al(OH)_3$) (Stumm and Morgan 1996). Because $Al(OH)_3$ is insoluble at pH between 4.5 and 8, Al concentrations in that pH range rarely exceed 1 mg/L (Watzlaf et al. 2004). When the MIW contains high concentration of dissolved sulfates, which is often the case, the precipitation of aluminum hydroxysulfate such as basaluminite ($Al_2(SO_4)(OH)_{10}$) or ettringite ($Ca_6Al_2(OH)_{12}(SO_4)_3 \cdot 26H_2O$) can be an important removal mechanism (Adams and Rawajfih 1977; Nordstrom 1982).

Manganese is a much more challenging metal to remove from MIW, especially in reducing conditions. Because manganese sulfide (MnS) has a high solubility product, it will only form when manganese concentrations are very high and several authors report that an excess of hydrogen sulfide is needed to effectively remove manganese as MnS (Johnson and Hallberg 2005b; Yoo et al. 2004). In addition, manganese weakly sorbs onto Fe-hydroxides (Willow and Cohen 2003). The removal of Mn through oxidation and hydrolysis processes and the subsequent precipitation of MnO_2 or MnOOH is limited by the slow oxidation of Mn^{2+} into Mn^{3+} or Mn^{4+} in acidic conditions. The abiotic oxidation is reported to be very slow if the pH is < 8, and the activity of microorganisms favoring biotic oxidation is limited to water with a pH > 6 (Watzlaf et al. 2004). Other removal mechanisms such as carbonate precipitation (e.g. $MnCO_3$) and adsorption onto organic matter are thought to play important roles in Mn removal in reducing systems (Bamforth et al. 2006; Robinson-Lora and Brennan 2010a; Trumm and Ball 2014; Younger et al. 2002). For example Venot et al. (2008) reported the precipitation of $MnCO_3$ in SRBR using crab-shell chitin in the substrate mixture where the median effluent pH was 6.9.

Depending on the metal's speciation, the pH, and the alkalinity (i.e. the bicarbonate concentration), the bicarbonate released during Eq. 2.12 (sulfate reduction) and/or Eq. 2.19 (carbonate dissolution) may react with some dissolved metals (e.g. Zn, Ni, Cu) to form insoluble metal carbonates (Eq. 2.21). Because this reaction occurs only if the pH is high (≈ 8 or higher) and the water net alkaline (Stumm and Morgan 1996), it is not expected to be an important removal mechanism in SRBR and is likely to occur only in microenvironments

within the SRBR substrate. Additionally, carbonate minerals are less stable than sulfide minerals.

$$M^{2+}(aq) + HCO_3^-(aq) \rightarrow MCO_3(s) + H^+(aq) \quad (2.21)$$

Divalent metal + bicarbonate → metal carbonate + proton

Table 2-3 and Figure 2-2 compare the solubility of some metal sulfides, hydroxides and carbonates.

Table 2-3. Theoretical solubility product constants (K_{sp}) of selected metal sulfides, hydroxides and carbonate in pure water at pH 7 and 25°C. Data from Hill et al. (2004).

	Sulfides	**Hydroxides**	**Carbonates**
Fe^{2+}	3.4×10^{-19}	8.9×10^{-16}	3.2×10^{-11}
Al^{3+}	-	1.3×10^{-33}	-
Mn^{2+}	2.1×10^{-14}	1.9×10^{-9}	1.8×10^{-11}
Zn^{2+}	2.3×10^{-25}	1.2×10^{-17}	1.4×10^{-11}
Cu^{2+}	6×10^{-37}	2.2×10^{-20}	1.4×10^{-10}
Ni^{2+}	3×10^{-19}	2×10^{-15}	6.6×10^{-9}
Cd^{2+}	8×10^{-28}	2.5×10^{-14}	5.2×10^{-12}

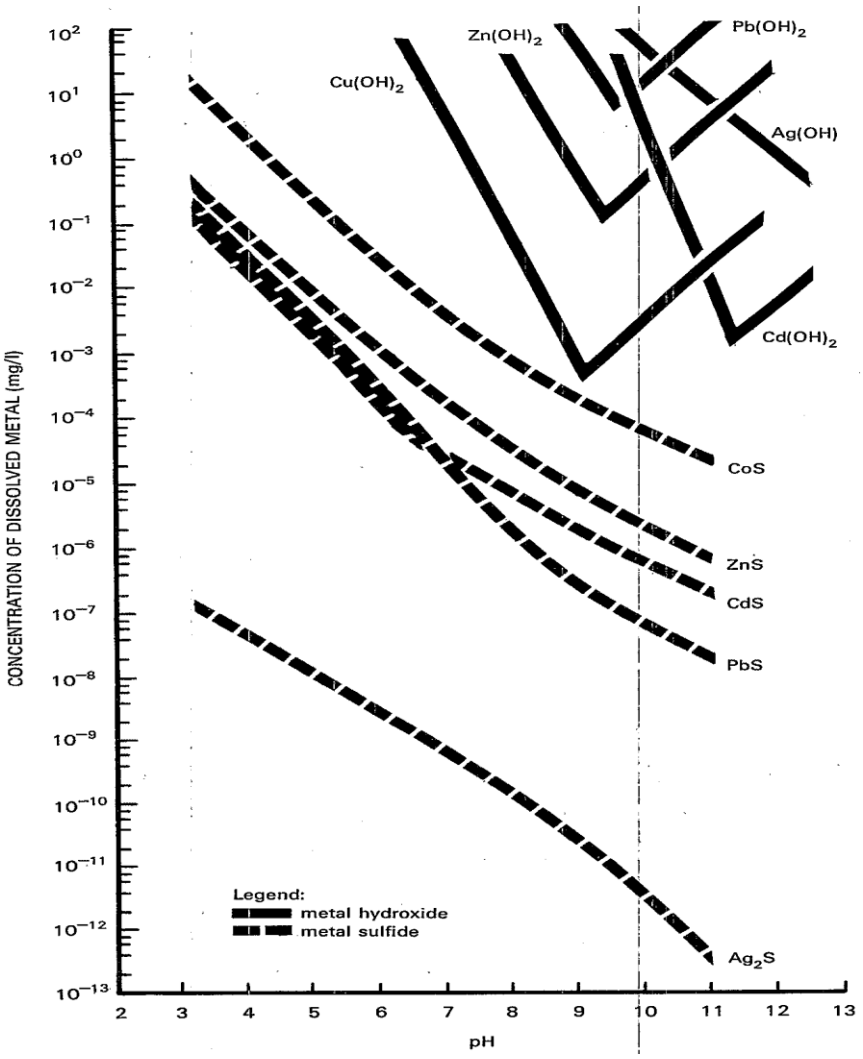


Figure 2-2. Solubilities of some metal sulfides and hydroxides as a function of pH and metal concentrations (US-EPA 1980).

It worth noting that the present discussion on metal removals applies to cationic metals only. Metals such as arsenic, selenium, antimony or molybdenum form anionic species in solution and their removal mechanisms are therefore very different from those of the cationic species discussed above. Because the concentrations of As, Se, Sb and Mo in the present study were very low (or below detection limits), no additional discussion on their specific removal mechanisms is presented here.

2.2.3.4 Performances of sulfate-reducing bioreactors

Since researchers first noticed the potential of sulfidogenesis to treat MIW, several generation of SRBR have been more or less successfully implemented around the world (Gusek 2013; Wildeman et al. 1993). Because these systems exhibit several advantages on other systems (e.g. SRBR can concurrently remove the key contaminants of metals, acidity and sulfate, they can handle net acidic MIW influents and rely only on waste materials) they are an attractive method to treat MIW. In addition, when properly designed, these systems can treat MIW containing high concentrations of both Fe and Al (most other passive treatments, especially aerobic and oxidizing systems, struggle to remove high concentration of Al without disturbing the proper functioning of the systems).

Because numerous substrate materials, including both simple and complex organic sources (Table 2-2), as well as various reactor design parameters have been tested (e.g. different HRT, temperature, influent pH, etc), direct comparisons between studies are often unpractical and great care must be taken when comparing results from different experiments. In addition, SRBR performances are not always reported with the same units (e.g. metal, sulfate and acidity removal can be reported in %, or mg/L/d or mol/m^3 of substrate/d), therefore adding more difficulties to the comparisons. Finally, some authors report removal efficiencies from day 1 onwards, when others report values only during steady-state operations (for example, it is a well-known fact that sulfate removal are generally high during the first few weeks of operation due to the sorption of SO_4^{2-} onto the organic matter, before it drops and reach a steady state behavior).

Table 2-4 summarizes the performance of various SRBR and vertical-flow wetlands treating MIW. These systems generally incorporated an alkaline material in their reactive mixture as it is becoming a standard practice in MIW treatment (Wildeman et al. 2006) . As it can be seen from Table 2-4, pH can be substantially increased to values ranging between 6 and 7, and metal removals (usually reported in % of metal removed) are typically high (> 90 %). Mn is usually poorly removed. Nevertheless, a few studies have high reported Mn removal (Robinson-Lora and Brennan 2009; Venot et al. 2008). These two specific studies used chitin-related products in the substrate mixtures. Chitin, which is a long-chain polysaccharide containing nitrogen, phosphates and soluble organic compounds, is present in various concentrations in the exoskeletons of crustaceans and various bivalves such as mussel shells, and has proven to be an efficient material for the removal of Mn (through both adsorption and possible precipitation of Mn-carbonates). On the contrary, sulfate reduction is usually less efficient and more variable than metal removal or increase in pH. Typically, sulfate removal ranged between 10 and 30 %, but values a low a 0 % or as high as 95 % have been reported.

Table 2-4. Performances of various field and lab-scale SRBRs and vertical flow wetlands used to treat MIW.

Substrates	HRT	Temp. (°C)	pH (influent/ effluent)	Metals removal	Sulfate removal	References
Mussel shells, wood waste, compost	1.95-11 days	14.7	~2.6/~6.7	95.9% for all metals; >99% Al	493-554 mg/L/d (~42%)	McCauley et al. (2009)
Spent mushroom compost, oak chips, wastepaper sludge, organic-rich soil	20 days	25	6.8/~7.5	<0.1ppm Cu & Zn; <100 ppm for Fe	~50%[c]	Chang et al. (2000)
Municipal compost, limestone, river sediments	16 hours	n.r.	3/6.8	15-75% Zn, >86% Cu; Al below detection limit	no sulfate reduction observed	Gibert et al. (2005)
Spent mushroom compost, crab-chitin	Batch exp.	20	2.95/6.7	100% Al; 93-97% Fe; 73% Mn	17.8 mg/L/d	Robinson-Lora & Brennan (2009)
Maple wood chips, sawdust, leaf compost, poultry manure	7.3-10 days	18-27	2.89/~6.5	60-80% Fe; >99% for Cd, Ni, Zn	~25-30%[c]	Neculita et al. (2008b)
Sand and lactate	16.2 hours	25	4.52/7.3	>97.5% for Cu, Zn, Ni; 82% Fe, 77.5% As	475 mg/L/d	Jong & Parry (2003)
Sheep manure and limestone	2.4-9 days	n.r.	2.4/6.3-7.7	Use of synthetic water without metals	7-24 mg/L/d	Gibert et al (2004).

Table 2-4. (cont.)

Substrates	HRT	Temp. (°C)	pH (influent/ effluent)	Metals removal	Sulfate removal	References
Compost and limestone	n.r.	n.r.	2.4/5.2-7.1	90% Fe, > 99% Al	0.25-0.35 $mol/m^3/d$ (~20%)	Thomas & Romanek (2002b)
Spent mushroom compost with pulverized limestone (10-15 wt. %)	5-17 days	18-24	3.2/6.4-7.1	~85% Fe, > 99% Zn & Al	0.21-0.33 $mol/m^3/d$ (~18-21%)	Dvorak et al. (1992)
Corn stover and walnut shells, limesone	5 and 10 days	n.r.	5.2/6.5	> 95 % Zn	0.13-0.3 $mol/m^3/d$	Figueroa et al. (2007)
Wood chips, dairy manure, hay and limestone	n.r.	2-5	6/7.2	> 95 % Cd, Cu, Pb, Zn, 1% Fe, 12% Mn	~ 50-80 %	Reisman et al. (2008)
Gravel, sand and ChitoRem™ (chitin related product)	4.5-7.5 days	4.6-9.2	4.8/6.4	> 95% Fe, Zn, Cu; 80% Mn	50-95%	Venot et al. (2008)

n.r. not reported

CHAPTER 3

SUBSTRATE MATERIAL CHARACTERISTICS & EXPERIMENTAL DESIGN

3.1 Introduction

The objective of this chapter was to characterize the organic and alkaline materials to be used in the SRBR. It included geotechnical and hydraulic measurements to determine how to achieve the desired hydraulic retention times and to ensure that an adequate flow could be maintained throughout the treatability study. Additionally, chemical and mineralogical analyses were performed to evaluate if deleterious concentration of metals could leach from the substrates. This chapter also presents the experimental design of the flow-through treatability study. Unless stated otherwise, all analyses were conducted in the Environmental Laboratory of the Civil and Natural Resources Engineering Department at the University of Canterbury.

3.2 Materials and Methods

3.2.1 Substrate materials and mixtures

The choice of an effective and economically viable substrate mixture is critical in the design of sustainable SRBR (Zagury et al. 2005; URS 2003; Neculita et al. 2007b; Gusek 2002). Generally, an effective complex substrate mixture comprises both organic and alkalinity generating materials. The key purposes of the substrate are to provide a suitable supply of

organic carbon for the microbial community to thrive, as well as an adequate acidity mitigating potential. Studies showed that using a combination of organic materials resulted in a better treatment efficacy compared to relying on a single substrate material (Cocos et al. 2002; Zagury et al. 2006). Furthermore, appropriate hydraulic conductivity and porosity of the substrate are required to achieve the desired hydraulic retention time, as well as to prevent clogging and the development of preferential flow-paths, which are reported as one of the main failure reasons in pilot and full scale passive treatment systems (Gusek 2004; Wildeman et al. 2006; Younger et al. 2002). Early bioreactors design utilizing exclusively compost and/or manure as reactive substrate had reported hydraulic conductivities in the order of 10^{-5} to 10^{-6} cm/sec (Hutchinson and Nairn 2005; URS 2003). Based on the observation that many of these early systems had hydraulic failures, Gusek (2002) and others recommended including more bulky materials such as bark or wood chip into the reactive mixtures. Since this practice has become conventional, less hydraulic failures have been reported.

Figure 3-1 shows the materials used in this study, organic materials included: (1) *Pinus radiata* bark (i.e. Monterey pine), (2) *Cordyline banksii* bark mulch (i.e. forest cabbage tree), and (3) a blended compost comprising of *Pinus radiata* bark, animal effluents, and pig manure sawdust. Alkaline materials included: (1) waste mussel shells or (2) limestone. Sub-rounded riverbed gravels were used in the bottom and top sections of the reactors. Organic materials, limestone and gravels were obtained from ParkHouse Garden Supplies (Christchurch, New Zealand). Mussel shells were donated by Solid Energy Ltd., and were originally sourced from a seafood industry located in Nelson, New Zealand. The mussel shells had been stockpiled outside for about one year previous to their use in the SRBRs, and were therefore subjected to

mechanical and chemical weathering. The weathering of the shells has at least one implication with regards to their use in SRBR; most of the labile and easily available organic carbon associated with the shells (e.g. seaweed and mussel meat remnants) has been washed out and is therefore not available for the SRB to conduct sulfidogenesis. In addition, the advanced weathering of the shells could have an influence on the calcium carbonate dissolution within the SRBR. Emmanuel and Levenson (2014), who studied limestone weathering rates, suggested that chemical weathering along fine-grain boundaries within a mineral structure facilitated the mechanical detachment of small particles, therefore increasing the surface area of the material, thus its rate of dissolution.

Substrate material proportions were determined from the literature review and previous studies conducted at the University of Canterbury and by other partners in New Zealand (Crombie et al. 2011; McCauley et al. 2009; McCauley et al. 2008; Trumm and Ball 2014), as well as from chemical and mineralogical analyses (e.g. metal content, carbon to nitrogen ratio). These proportions were chosen to provide a combination of easily biodegradable and labile substances (often reported as easily available substances in the literature, EAS) especially important upon the start-up period of newly operating bioreactors, and more recalcitrant substances like cellulose and hemicellulose that would sustain the microbial communities over the long-term treatment period (Gibert et al. 2004). Alkalinity generating materials were included to a relative volume of 30 % to provide acidity mitigation. The two alkalinity materials (mussel shells or limestone) were tested separately in the SRBR to determine their potential differences in MIW treatment efficiency, especially their influence on the alkalinity generation and the metal removal efficiencies (Figure 3-2).

Figure 3-1. Organic and alkaline materials used in the bioreactor substrates: (A) bark, (B) bark mulch, (C) compost, (D) gravel, (E) mussel shell, (F) limestone.

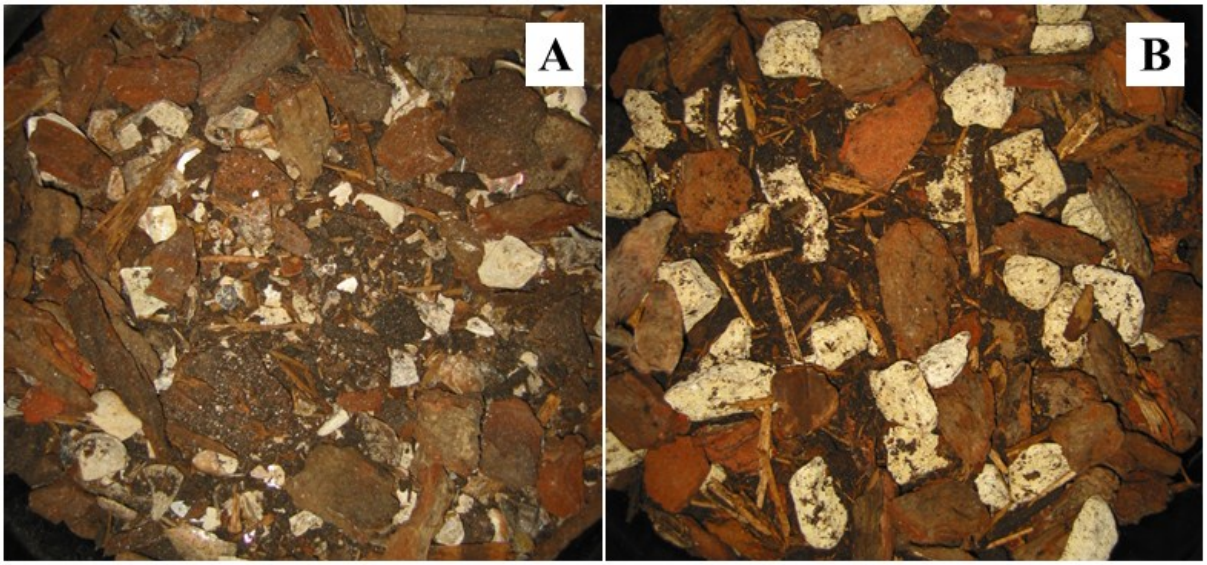


Figure 3-2. Substrate mixtures used in the SRBR: (A) mussel shell mixture, (B) limestone mixture.

3.2.2 Geotechnical and hydraulic analyses

3.2.2.1 Moisture content

The moisture content (M_c) expresses the relative amount of water contained in a material. It was determined for each individual substrate materials as received from the suppliers (i.e. wet material). Duplicate samples of the materials were weighted, dried in an air-oven at 105°C until constant weight was reached, cooled in a desiccator, and reweighted. Moisture content expressed on a wet basis was computed using Equation 3.1.

$$M_c = ((m_{wet}-m_{dry}) / m_{wet}) \times 100 \quad (3.1)$$

Where:

M_c = moisture content (%),

m_{wet} = mass of sample wet (g) (i.e. as received from the supplier),

m_{dry} = mass of sample dry (g).

3.2.2.2 Volatile and fixed solids

Volatile solids, as defined by US-EPA method 1684 (US-EPA 2001), correspond to the mass loss after ignition while the fixed solids constitute the residues left in the vessel after ignition (the remaining ashes). The volatile and fixed solid contents were determined for each substrate material as received from the suppliers. Duplicate samples of the materials were weighed, dried in an air-oven at 105°C to remove the water fraction (i.e. moisture content) until constant weight was reached, cooled in a desiccator and then placed in a muffle furnace at 550°C for at least four hours (or until constant weight was reached), cooled in a desiccator and reweighed. Duplicate analyses were repeated until measurements agreed within 5 %. Equation 3.2 was used to calculate the volatile solids and equation 3.3 to calculate the fixed solids. The results can be expressed as % of solids or as mg of solids per kg of dry sample.

$$\text{Volatile solids (\%)} = (m_{total} - m_{volatile} / m_{total} - m_{dish}) \times 100 \quad (3.2)$$

$$\text{Volatile solids (mg/kg)} = (m_{total} - m_{volatile} / m_{total} - m_{dish}) \times 1{,}000$$

Fixed solids (%) = ($m_{volatile}$ - m_{dish}/ m_{total} – m_{dish}) x 100 (3.3)

Fixed solids (mg/kg) = ($m_{volatile}$ - m_{dish}/ m_{total} – m_{dish}) x 1,000

Where:

m_{total} = mass of dried residue and dish (g) (i.e. after drying at 105°C),

$m_{volatile}$ = mass of residue and dish after ignition (g) (i.e. after ignited at 550°C),

m_{dish} = mass of dish (g).

3.2.2.3 Bulk density

Bulk density (ρ) was determined for each individual substrate material as received from the suppliers. It was determined by weighing each material and dividing the mass by a known total volume (volume of substrate and volume of pore spaces) as shown in Equation 3.4. Measurements were conducted in triplicate.

$$\rho = m_s / V_t \quad (3.4)$$

Where:

ρ = bulk density (-),

m_s = mass of material (kg),

V_t = total volume (m^3).

3.2.2.4 Saturated hydraulic conductivity

The saturated hydraulic conductivity (K_s), often expressed in cm/sec, is a property that describes the ease with which water can flow through a saturated porous media. It is influenced by porosity, grain-size and pore-size distribution, void ratio, fluid viscosity (hence the fluid temperature) and the degree of saturation of the media (Das 2002). The hydraulic conductivity of unsaturated soils is less than saturated soils and increases rapidly with the degree of saturation. It also greatly differs between soil types or mixed substrate compositions, and it is therefore an important parameter to assess before engineered SRBR systems are constructed to ensure that adequate flow-through rates can be achieved during the MIW treatment.

In this study, the hydraulic conductivity was measured on the two reactive mixtures (e.g. the mixture containing the organic materials plus the mussel shell and the mixture containing the organic materials plus the limestone) and not on the individual materials. Each mixture was first soaked for 24 h in order to provide a more stable and consistent flow (Bolis et al. 1992). They were then drained and re-saturated upwardly with tap water before conducting the hydraulic conductivity measurements in triplicate using the constant-head method (Figure 3-3), and computed using a derivation of Darcy's law (Equation 3.5).

$$K_s = q \times L / A \times \Delta h \qquad (3.5)$$

Where:

K_s = saturated hydraulic conductivity (m/s),

q = flow rate of water through the saturated substrate (m^3/s),

L = distance between standpipe manometers (m),

A = cross-sectional area of the substrate (m^2),

Δh = head difference between the manometers (m).

The hydraulic conductivity is typically reported at 20°C. Therefore, if the temperature is different from 20°C during the test, it should be corrected using a correction factor (Table 3-1) as shown in Equation 3.6.

$$K_{20°C} = \eta T \times K_{T°C} \quad (3.6)$$

Where:

$K_{20°C}$ = Ks at 20°C,

ηT = temperature correction factor,

$K_{T°C}$ = Ks at the actual water temperature measured in the experimental set-up.

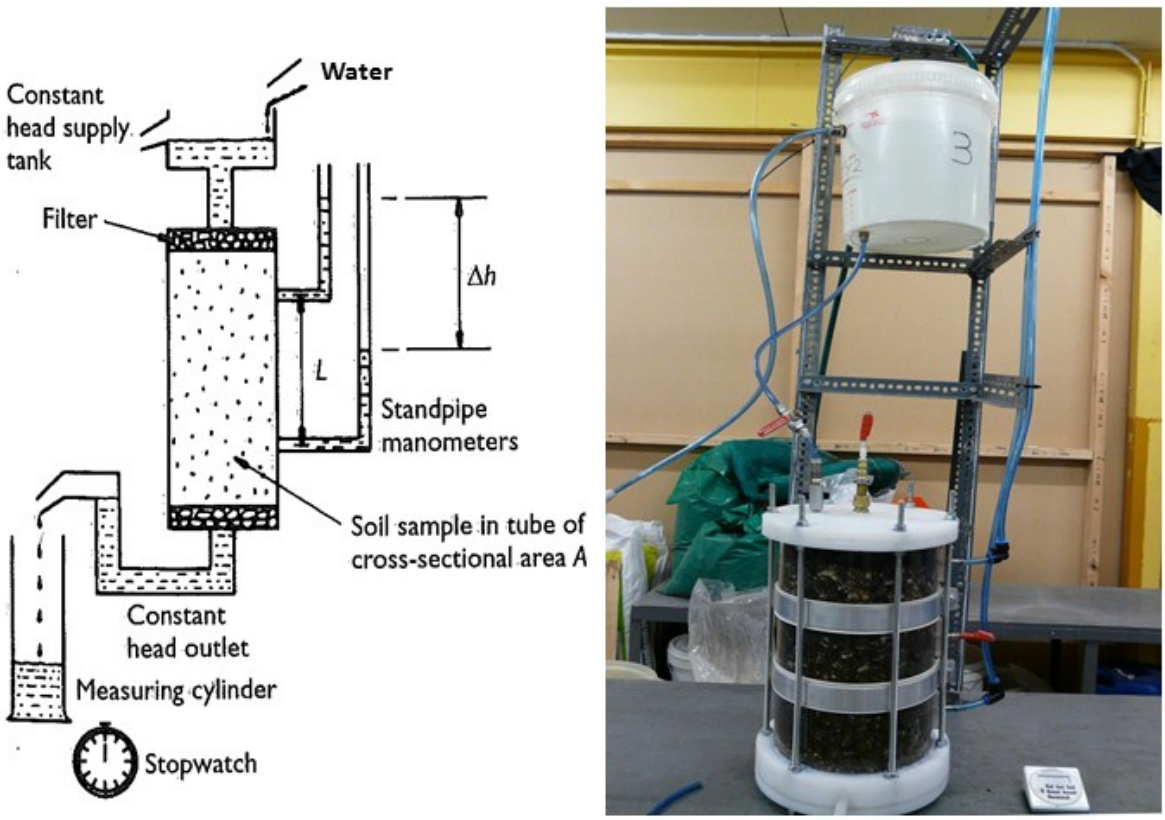


Figure 3-3. Constant head permeameter schematic (Powrie 2004) and experimental set-up.

Table 3-1. Temperature correction factor (Das 2002).

Temperature (°C)	ηT	Temperature (°C)	ηT
15	1.135	23	0.931
16	1.106	24	0.910
17	1.077	25	0.889
18	1.051	26	0.869
19	1.025	27	0.850
20	1.000	28	0.832
21	0.976	29	0.814
22	0.953	30	0.797

3.2.2.5 Porosity

Total porosity (n) represents the ratio of pore volume (i.e. void volume; voids can be occupied by air, water or a combination of both) to the total volume of a sample, and is calculated using Equation 3.7.

$$n = V_V / V_T \quad (3.7)$$

Where:

n = total porosity (-),

V_V = volume of void (m^3),

V_T = total volume of substrate (m^3).

The total porosity is the sum of the air porosity and the water content. If a sample is saturated and allowed to drain, the water content after drainage is called the water holding capacity. For this study, the effective porosity is defined as the difference between the total porosity and the water holding capacity and represents the fraction of voids occupied by the free draining liquid (e.g. where the flow actually occurs). It is a crucial factor to consider when calculating the HRT_n.

In this study, the effective porosity of the two reactive mixtures was assessed following a simple methodology previously used by Drury (1999). Briefly, the reactors were filled

upwardly (to avoid any air pocket forming within the substrate mixture) with tap water, sat covered for 24 h before being drained (downwardly) by gravity. Using Equation 3.8, the volume of water drained from each layer (e.g. top and bottom gravel layers, and substrate mixture layer) divided by the corresponding layer's volume gave the effective porosity. The measurements were conducted in duplicate.

$$n_e = V_W / V_T \quad (3.8)$$

Where:

n_e = effective porosity (-),

V_W = volume of water (m^3),

V_T = volume of substrate (m^3).

3.2.2.6 Hydraulic retention time

Theoretical (i.e. nominal) hydraulic retention time (HRT_n) was calculated using Equation 3.9 derived from Darcy's Law (Younger et al. 2002). For each of the four systems, individual HRT_n were calculated for the gravel layers (bottom and top) and for the substrate mixture layer, and then summed to obtain a total HRT_n for each of the four SRBR systems (see section 3.4.1 and Figure 3-6).

$$HRT_n = V \times n_e / Q \quad (3.9)$$

Where:

V = layer volume (m^3),

n_e = effective porosity of the corresponding material (-),

Q = inflow (m^3/day).

3.2.3 Chemical and mineralogical analyses

All chemical reagents used were analytical grade, and all glassware was previously acid-washed in 10 % HNO_3 for a minimum of 24 h, and rinsed three times with tap water and three times with deionized water. All metal samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent-7500cx), according to Standard Methods 3125B (APHA 2005), in the Chemistry Department at the University of Canterbury. ICP-MS quality assurance and quality control (QA/QC) was achieved through the use of accredited standards (trace elements in water, NIST 1643e), blanks (i.e. deionized water), and replicate analyses (10 %)[1].

3.2.3.1 Total metal contents

Total metal concentrations in substrate materials were measured to screen for any metal present in concentrations that might be deleterious to the overall treatment function of the SRBR. All materials were digested individually following the hot HNO_3-HCl digestion method 3030F (APHA 2005). Briefly, 1 ± 0.005 g of sample was weighed into an acid-washed

[1] 10 % means that one blank and one replicate sample were analyzed every ten samples. In addition to the National Institute of Standards and Technology (NIST) standard used for instrument calibration and recovery checks, one spiked samples was analyzed every 20 samples as a specific in-run recovery check (using Rh).

beaker and digested using concentrated HNO_3 (Fisher analytical grade, 69 %) and concentrated HCl (Fisher analytical grade, 36 %). The samples were placed on a hot plate, covered with a watch glass, and digested at approximately 100°C until the digestate colors remained constant. Cooled samples were filtered using Whatman paper #541 and made up to 100 ml using deionized water. The digestions were further diluted and analyzed for metal concentrations by ICP-MS. The metal concentrations were then computed using Equation 3.10 and reported on a dry weight basis.

Relative metal amount (mg/kg) in dry solid sample = ((C x V) / M) x moist. (3.10)

Where:

C = concentration in the acid extract (mg/L),

V = volume of extract (L),

M = mass of sample aliquot extracted (kg),

Moist. = moisture content (%).

3.2.3.2 Total organic carbon and nitrogen

Total organic carbon and total nitrogen contents, as well as carbon to nitrogen ratios (C/N) were measured on all organic materials by RJ Hill Laboratories Ltd. (an IANZ[2] accredited laboratory located in Christchurch, New Zealand) following the Dumas combustion method (Hill 2014). Briefly, the samples were dried (105°C for 24 h), ground and sieved (< 2 mm)

[2] International Academy of New Zealand (accreditation body)

before being combusted at 900°C in a closed chamber with oxygen and the resultant gas (CO_2 and N_2) determined by a thermal conductivity detector.

3.2.3.3 Total sulfur

Total sulfur was measured on all organic materials by RJ Hill Laboratories Ltd. following a hot HNO_3-HCl digestion (based on US-EPA method 200.2) and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES).

3.2.3.4 Mineralogical analyses

Mineralogical composition of the alkaline materials was determined by X-ray diffraction (XRD) and X-ray fluorescence (XRF) by CRL Energy Ltd. staff (CRL Environmental Laboratory in Lower Hutt, New Zealand, is an IANZ accredited laboratory). Duplicate samples were oven dried at 110°C overnight, ground in a ring mill and prepared as unoriented powder mounts. The diffractometer (Philips PW1729) was equipped with a graphite monochromator and a Co anode X-ray source and the analyses were performed from 5 to 80° (2θ) at a 1° (2θ) per min scanning time. Phase identification and quantification were carried out by a Siroquant search and match program. The fluorescence spectrometer (Siemens SRS3000), equipped with a 3kW Rh end-window X-ray source, was used to determine major oxides (detection limit 0.01 weight %) and trace elements (detection limit 0.001 wt. %) with a SpectraPlus software (version 1.7). Loss on ignition (LOI) was determined gravimetrically in a muffle furnace (detection limit 0.01 wt. %).

3.3 Experimental design and reactor characteristics

Four SRBR treatment designs (operated in duplicate) were chosen to investigate the influence of two different alkalinity sources (mussel shells or limestone) and two different hydraulic retention times (short and long HRT). The four systems were: mussel-shell short HRT (MS-S), mussel-shell long HRT (MS-L), limestone short HRT (LS-S), and limestone long HRT (LS-L). Figure 3-4 shows the experimental set-up and a detailed schematic of one reactor. Figure 3-5 shows the actual laboratory set-up. Figure 3-6 shows a schematic of one reactor with the volume of each section as used to calculate the nominal HRT_n.

All reactors were 45 cm high and 31 cm wide high density polyethylene (HDPE) cylinders with rubber-seal screw lids. One pore-water sample port was positioned 14 cm above the inlet port. Each reactor was filled with clean sub-rounded gravels (washed in distilled water) to a depth of 8 cm to allow a uniform dispersion of the influent MIW (flow-equalization layer). A 31 cm layer of reactive substrate mixture was placed above this layer and a 3 cm layer of clean sub-rounded gravel was placed on top of the reactive mixture to maintain the substrate in place. A fine mesh geotextile separated the reactive substrate from the bottom and top gravel layers to prevent washout of fine substrate materials and clogging of the inlet and outlet ports (geotextile was also placed on the port-water sample port inside the reactor to prevent blockage). The MIW was fed into the bottom of each reactor using split peristaltic pumps and treated discharge freely drained from the effluent port situated on the upper part of the reactors. Effluent samples were collected in 500 ml polypropylene (PP). The upward-flow design was chosen over more conventional downward flow to abate the development of preferential flow paths, minimize compaction of the substrate mixtures, and to help maintain

an adequate permeability (URS 2003). The substrate reactive mixture compositions are shown in Table 3-2 (volumetric basis) and Table 3-3 (weight basis).

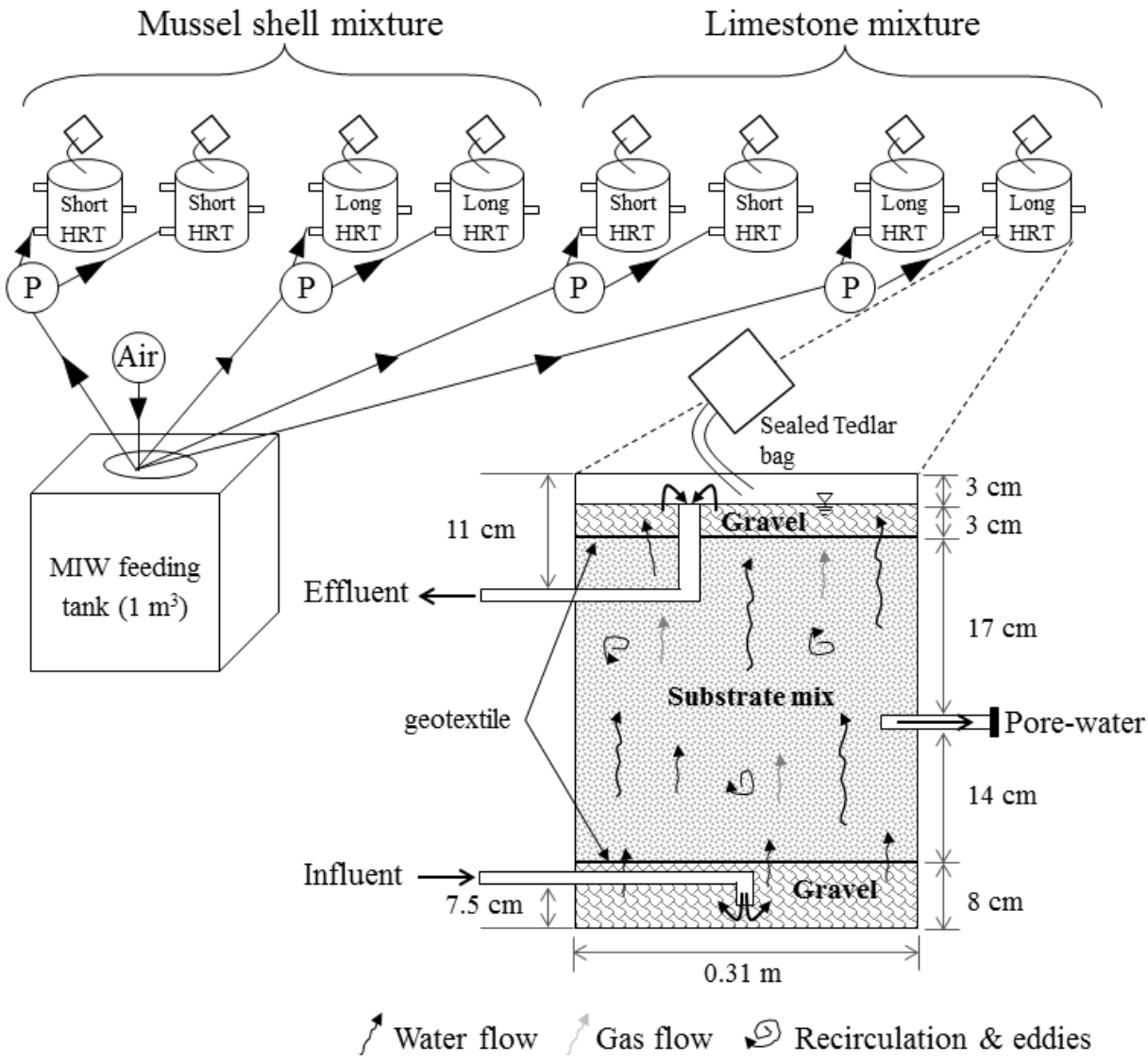


Figure 3-4. Experimental set-up and design of a reactor showing water and gas flows.

Figure 3-5. Laboratory set-up.

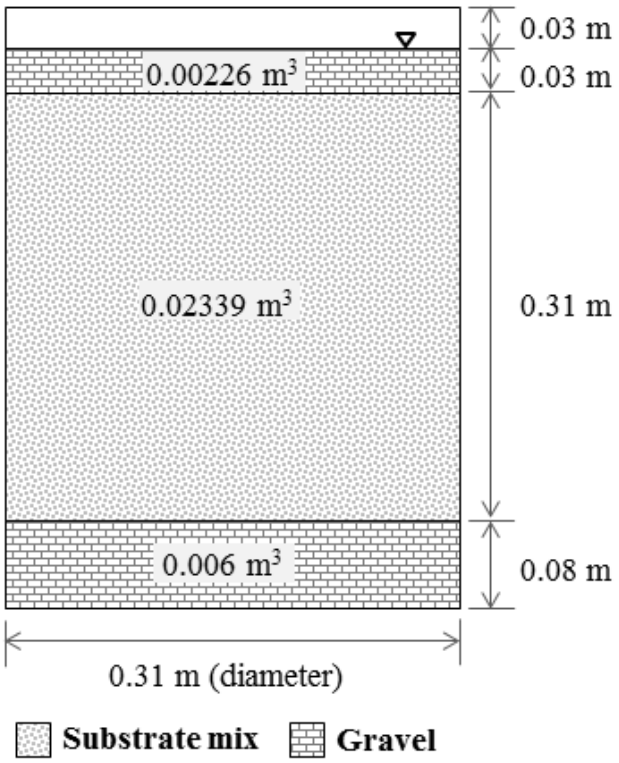


Figure 3-6. Reactor schematic showing volume of each section.

Table 3-2. Substrate mixture compositions on a volume basis (v/v %).

	Mussel shell mixture	**Limestone mixture**
Bark (*P. radiata)*	30	30
Bark mulch (*C. banksii)*	20	20
Compost	20	20
Mussel shell	30	-
Limestone	-	30
Total (%)	100	100

Table 3-3. Substrate mixture compositions on a weight basis (kg and wt. %).

	Mussel shell mixture	Limestone mixture
Bark (*P. radiata)*	2.43 (18.5 %)	2.43 (14.9 %)
Bark mulch (*C. banksii)*	0.89 (6.8 %)	0.89 (5.4 %)
Compost	3.21 (24.4 %)	3.21 (19.6 %)
Mussel shell	6.61 (50.3 %)	-
Limestone	-	9.82 (60.1 %)
Total (kg)	13.14 (100 %)	16.35 (100 %)

Weight of the materials as received from the supplier.

Before the start of the treatability experiment, the reactors were upward-flow saturated with MIW (from MIW batch #1, Table 4-2, Chapter 4) and operated in batch mode for a 3-week period. This acclimation stage was necessary to create a favorable reducing environment and to allow the establishment of an effective bacterial population capable of sustaining sulfate-reduction rates. The sulfate-reducing bacteria naturally present in the compost self-inoculated the reactors (i.e. no system was purposely inoculated). The time period of three weeks was based both on recommended values found in the literature (Neculita et al. 2008a) and on measured parameters (i.e. oxido-reduction potential, sulfate, and sulfide concentrations) in effluent and pore water samples (Appendix A-1). Following this acclimation period, the MIW was pumped into the reactors following a semi-continuous upward-flow approach (short HRT systems received influent MIW at a flow of 50 ml/min for 15 minutes 6 times a day, while long HRT systems received 50 ml MIW per min for 15 min twice a day). Before being conveyed into the SRBR, the influent MIW was continuously aerated with an air pump to simulate the aerobic and oxidizing conditions measured at the coal mine site where the MIW

was sourced. The four systems were operated in duplicate (eight reactors in total) for a first continuous period of 20 weeks (March through July 2013). After the 5-month experiment, all the reactors were stopped for two months during which the preliminary data was studied. The reactors were kept saturated with MIW during the interruption phase, and all feeding and sampling ports were kept closed to prevent the introduction of oxygen into the systems. After this interruption period, the four systems were re-started for a second 21-weeks experiment (October 2013 to March 2014). During the second phase, only one reactor for each system was operated (4 reactors in total; the best performing reactor of each pair was selected based on the data obtained during the first 5-month experiment). The 2-month interruption period could have had positive effects on the bioreactor performances. For example, Eger and Wagner (2002) reported temporarily increase in sulfate-reduction rates when bioreactors containing compost were allowed to rest for a period of time, and Whitehead et al. (2005) observed that the effluent pH at the Wheal Jane remediation system increased from approximately 5.5 to values between 6 and 7 after a resting period of 4 months. Additionally, several authors reported that resting periods were beneficial to decrease the risk of bio-clogging in vertical-flow wetlands and to restore the hydraulic conductivity (Hua et al. 2014; Kadlec and Wallace 2009). The whole flow-through treatability experiment was conducted at air room temperature (monitored at 12-19°C).

The original experimental design included Tedlar® bags fixed on top of the reactors to capture the excess of hydrogen sulfide gas produced by the microbial sulfate reduction (unreacted H_2S; the upward-flow design was also chosen to facilitate the gas transfer into the Tedlar® bags). Due to the nature of H_2S gas being relatively heavy and soluble (Carroll and Mather

1989), most of the gas exited the reactors through the effluent port. Even after the addition of a P-shape gas trap on the outlet pipes, a considerable amount of gas continued to exit the reactor through the effluent port, rendering its capture and quantification unfeasible. Nevertheless, seven gas samples were collected using the Tedlar® bags and analyzed by gas chromatography by CRL Energy Ltd. staff (CRL Environmental Laboratory, Lower Hutt, New Zealand). Surprisingly and despite the fact that a strong hydrogen sulfide odor was present in the laboratory during all sampling events, no hydrogen sulfide was detected in any of the seven gas samples (Appendix A-4). It should also be noted that air entered the bioreactors during some sampling events (e.g. the pore-water sampling ports sometime acted like siphons and sucked air into the reactors). This ingress of oxygen happened at least once for each reactor during the first 5-month treatment period, but more frequently during the second 5-month period, especially to reactor MS-S. The ingress of oxygen likely resulted in less reduced environments within the reactors and may even have led to the development of oxic microenvironments, especially near the pore-water sampling port inside the SRBR.

3.4 Results and Discussion

3.4.1 Geotechnical and hydraulic properties

Results from the geotechnical and hydraulic analyses are summarized in Table 3-4 and Table 3-5.

Table 3-4. Geotechnical parameters of individual materials used in the reactive substrates. Values are average (n=2) or median (n=3).

	Bark	Bark mulch	Compost	Mussel shell	Limestone
Size[a] (mm)	30	40 (L) 10 (W)	-	5-20	20-40
Moisture (%)	39.6	18.1	50.8	12.6	- [b]
Volatile solids (%)	96.9	91.6	50.3	4.5	- [b]
Fixed solids (%)	3.1	8.4	49.7	95.5	-
Bulk density (kg/m^3)	345.7	191.1	687.1	942.9	1399.8

[a] Sub-rounded gravels were 20-50 mm in diameter; L = length, W = width;
[b] moisture and volatile solids content for the limestone were assumed to be 0 %.

The moisture content and the bulk density are important parameters because they are used to (1) convert the results (e.g. metal contents) from a wet matter basis to a dry matter basis; and (2) to convert substrate compositions from a volumetric to weight basis. These conversions are important because they allow a better comparison between studies (e.g. the moisture content can vary greatly depending on how and where the materials have been stored, and therefore influence the material masses and substrate compositions on a weight (%) basis). In addition, the design of SRBR substrates is often done on a volumetric basis (easier and more reproducible), but the bulk materials are usually ordered from the suppliers on a weight basis.

The bulk density of the shells was approximately 1.5 times lower than that of the limestone, a difference likely explained by the specific shapes (i.e. concave wedge-shape resulting in a higher density of pores) and the elemental composition of each material. Similarly, the bulk densities of the organic materials were lower than that of the alkaline materials, which is mostly due to the difference in organic/inorganic contents (i.e. the volatile solids).

Table 3-5. Hydraulic parameters of the reactive substrates. Values are average (n=2) or median (n=3).

	Mussel shell mixture		Limestone mixture	
	short HRT	**long HRT**	**short HRT**	**long HRT**
Porosity gravel	0.38			
Porosity mixture	0.51		0.49	
Ks (cm/sec)	6.29×10^{-2}		9.72×10^{-2}	
Inflow (L/day)	4.5	1.5	4.5	1.5
HRT_n gravel[a] (d)	0.7	2.1	0.7	2.1
HRT_n mixture (d)	2.6	7.9	2.5	7.6
HRT_n total (d)	3.3	10.0	3.2	9.7

[a] HRT gravel combined bottom and top sections.

The volatile solids are used as an approximation of the amount of organic matter present in a sample, and the fixed solids approximate the inorganic matter. As the methodology does not strictly distinguish between organic and inorganic matter (i.e. the ignition at 550°C also decomposes and volatilizes some mineral salts and therefore results in greater mass losses), it usually slightly overestimates the true organic content of the materials. The volatile and fixed solids results logically confirmed that the organic materials contained much more organic matter than the alkaline materials. The little organic matter associated with the mussel shells (4.5 %, Table 3-4) presumably included seaweed and mussel meat remnants, and organic

matter present within the shells. Due to its blended nature, the compost had approximately a 50/50 % composition between organic and inorganic matter.

Since suspended solids, metal precipitates, and biofilms can alter the hydraulic properties of the treatment systems (i.e. reduce the permeability), thus affecting their longevity and performance, it is crucial to ensure that both the hydraulic conductivity and the effective porosity of the substrate prior to the MIW treatment are within the recommended ranges. Due to the similar nature of the two reactive mixtures (i.e. only the alkaline material component changed), their effective porosities, measured at 0.51 and 0.49 for the mixture containing mussel shell and limestone respectively, are believed to be equivalent considering the limits of the measurement methods used. More importantly these values were well within the range of 0.35 - 0.63 recommended by Amos and Younger (2003) for lab-scale systems. The gravel effective porosity was calculated at 0.38 and corresponds to fine to medium gravels (Das 2002). The saturated hydraulic conductivities were 6.29×10^{-2} cm/sec and 9.72×10^{-2} cm/sec for the mixture containing the mussel shells and the limestone, respectively. These values were again well within the recommended range of 10^{-2} and 10^{-3} cm/sec to avoid plugging and clogging of the substrate mixture in passive SRBR (Neculita et al. 2007b; URS 2003).

The short HRT was chosen to be approximately 3 days, and the long HRT more than double the short. The 3 days value was based on the assumption that metal sulfide precipitation (in SRBR containing complex mixtures of organic materials) takes 3 to 5 days to occur and that efficient treatment of strongly contaminated MIW takes 4 or more days (Neculita et al. 2008a; URS 2003; Younger et al. 2002). Using the volume of each of the reactor's layers (Figure 3-6)

and the hydraulic parameters (Table 3-5), the nominal HRT_n of each section of the reactors (e.g. top and bottom gravel layers, reactive substrate layer) were computed using Eq. 3.10 and summed to obtain a total HRT_n for each of the fours systems (MS-S, MS-L, LS-S, LS-L). It resulted in two SRBR systems (one of each alkaline material) being operated at short HRT_n of 3.2 and 3.3 days for limestone and mussel shell reactors, respectively, and two SRBR systems (one of each alkaline material) being operated at long HRT_n of 9.7 and 10 days for limestone and mussel shell reactors, respectively. The differences in the calculated HRT_n for the systems containing mussel shells or limestone resulted from the differences measured between the two effective porosities. In reality, as the differences in the effective porosities are thought to be insignificant, the short and the long HRT_n are assumed to be similar for both mixtures and will be referred to as short (3 days) and long (10 days) HRT_n. The net inflow rates, chosen to achieve the desired HRT_n, were 4.5 L/day and 1.5 L/day for the short and the long HRT, respectively (Table 3-5). The actual inflow delivered to each reactor was verified every two weeks using a 100 ml graduated cylinder and a stopwatch.

3.4.2 Chemical and mineralogical properties

Table 3-6 shows the results from the hot acid digestions performed on the organic and alkaline materials prior to their use in the SRBR.

Table 3-6. Metal contents in the substrate materials. Values are in mg/kg of dry material (average of duplicate analyses).

	Bark	Bark mulch	Compost	Mussel shell	Limestone
Fe	7,009.06	897.69	10,629.63	765.12	4,233.93
Al	6,046.74	929.32	8,633.88	785.22	2,777.06
Mn	171.31	92.32	679.07	28.37	216.25
Zn	53.27	35.64	494.29	10.84	22.82
Ni	4.86	0.92	10.25	52.35	68.16
Cu	16.18	6.72	82.68	3.77	9.20
Pb	5.90	2.15	15.89	1.78	2.56
Cd	0.29	0.16	0.65	0.11	0.55

Overall, compost had the higher metal content of all materials, followed by the bark and the limestone. Bark mulch and mussel shells had lower and similar metal contents. Fe, Al and Mn constantly had the highest metal contents across all materials. Because Fe and Al were not expected to leach significantly from the reactors (SRBR have high reported removal rates for the two metals), and because Mn was found in relatively high concentrations only in the compost material, these results suggested that overall the organic and alkaline materials used in the SRBR substrates were unlikely to contribute to high metal concentrations in the treated effluents. Additionally, these results showed that the limestone had a higher metal content

compared to the mussel shell (between 1.4 times for Pb and 7.6 times for Mn more trace metals were contained in the limestone). Because these metals were contained within the calcium carbonate and the quartz mineral lattices (the limestone contained approx. 1 wt. % of quartz), it was considered unlikely that the limestone would contribute to a higher metal release in the SRBR effluents compared to the mussel shell. These specific findings and their impact on the water treatment efficacy and the post-treatment substrate autopsies are discussed in more details in Chapter 4 and Chapter 5.

Table 3-7 shows total organic carbon, total nitrogen, carbon to nitrogen ratio (C/N) and total sulfur contents, and Table 3-8 shows major cations contents (Ca, Na, Mg, K) in the substrate materials.

Table 3-7. Total organic carbon, total nitrogen, C/N, and total sulfur in the organic substrate materials. All values are in mg/kg of dry materials. C/N is dimensionless.

	Bark	**Bark mulch**	**Compost**
Total organic carbon	550,000	483,000	292,000
Total nitrogen	2000	3,200	10,800
C/N	280	150	27
Total sulfur	1,041	221	3,390

Table 3-8. Major cation contents in the substrate materials. All values are in mg/kg of dry materials.

	Bark	Bark mulch	Compost	Mussel shell	Limestone
Ca	0	0	802.87	365,142.18	363,680.06
Na	35.08	11.03	359.30	5,348.03	260.98
Mg	546.30	54.85	2227.65	481.86	3,751.49
K	203.78	94.24	2110.38	319.49	722.94

As discussed in Chapter 2, C/N values are important to consider when selecting the organic materials to be used in a reactive substrate mixture. In the present study, the values for the individual materials are slightly out of the recommended range of 45 - 120 defined by Okabe et al. (1992) and far above the range of 6 to 10 recommended by Prasad et al. (1999). Values above these thresholds are reported to be too low in nitrogen (or too high in carbon) to properly stimulate SRB growth and to offer suitable biodegradation rates of complex organic substrates. However, it worth noting that Okabe et al. (1992) used lactate in their study, which is a readily available carbon source for the SRB compared to the more recalcitrant carbon sources used in the present study and in the study of Prasad et al. (1999). In addition, Okabe et al. (1992) focused on the SRB *Desulfovibrio Desulfuricans* and not on a mixed culture. Finally, great care must be taken when comparing C/N values between studies. Indeed, some authors used total carbon and total nitrogen values while other used BOD or COD to estimate the carbon content and Total Kjeldahl Nitrogen (TKN) to report the nitrogen content. Therefore it is not surprising to find that studies successfully used materials with C/N values as low as 6.5 (activated sludge) or as high as 460 (wood materials) (Prasad et al. 1999; Zagury et al. 2006). In the present study, the carbon contents correspond to the Total Organic Carbon

(TOC) and not to a biologically available carbon. Therefore a certain amount of organic carbon present in the materials used in the present study was hardly biodegradable and not directly available to the SRB, and the true C/N values, although hard to evaluate, were probably lower than the values presented in Table 3-7. In addition, using the weight of the materials (Table 3-3) and the C and N contents for each individual material (Table 3-7), a total C/N ratio can be estimated for the whole organic portion of the mixtures. As the amount of organics was similar in both mixtures (mussel shell and limestone mixtures differed only by their alkaline material contents), a single value of 63.8 was found. While this value was well within the recommended range of 45 - 120, it was still above the value of 10 suggested by Prasad et al. (1999), possibly indicating that the organic mixture was too low in nitrogen to properly stimulate the SRB growth and the biodegradation of the substrate.

Based on the calcium and carbon contents present in the substrate materials (Table 3-7 and Table 3-8), the weight of the materials (Table 3-3), as well as the calcium carbonate dissolution (Eq. 2.17) and the sulfate reduction equations (Eq. 2.11 and 2.12), a total potential alkalinity generation was computed (Table 3-9). This total potential alkalinity calculation assumed that all the substrate materials would be completely consumed over time and is based on the following assumptions: (1) all Ca in the mussel shells or the limestone was present as calcium carbonate and would entirely dissolve to produce bicarbonate; (2) any Ca present in the organic materials did not contribute to alkalinity generation (e.g. any gypsum present in the compost would not generate alkalinity); (3) all C in the organic materials was present as organic carbon and would be entirely converted to bicarbonate; (4) any organic C present in the alkalinity materials would not contribute significantly to the alkalinity generation.

Table 3-9. Total potential alkalinity generation for the actual mixtures containing mussel shell or limestone. Total alkalinity generation in kg of $CaCO_3$ per m^3 of substrate and relative contributions (%) in brackets.

	Mussel shell mixture	Limestone mixture
Calcium carbonate dissolution	257.8 (21.1 %)	381.2 (28.4 %)
Sulfate reduction	963.1 (78.9 %)	963.1 (71.6 %)
Total	1220.9 (100 %)	1344.3 (100 %)

Results indicated that that total potential alkalinity generation per cubic meter of substrate ranged between 1220 and 1344 kg of $CaCO_3$ equivalent per cubic meter of substrate. The sulfate reduction would account for approximately 70 to 80 % of the total alkalinity generation, while the calcium carbonate would account for the remaining 20 to 30% (Table 3-9). In the mussel shell system, the calcium carbonate dissolution had a slightly lower theoretical capacity of alkalinity production compared to the limestone containing system. This difference is simply explained by the fact that the mussel shell reactors contained less alkaline material compared to the limestone reactor (on a weight basis). Subsequently, the same total theoretical alkalinity generation potential was calculated for hypothetical systems containing the exact same mass of mussel shell or limestone. These results confirmed that the sulfate reduction mechanism would yield a higher amount of alkalinity (75.5 - 75.6 %) compared to the calcium carbonate dissolution (24.4 - 25.5 %). This last observation is of course valid only if both alkaline materials have very similar $CaCO_3$ concentrations, as it is the case in the present study (see XRF results below).

Results from the X-ray fluorescence (XRF) analyses performed on the alkaline materials are presented in Figure 3-7 (major oxides) and Figure 3-8 (trace elements).

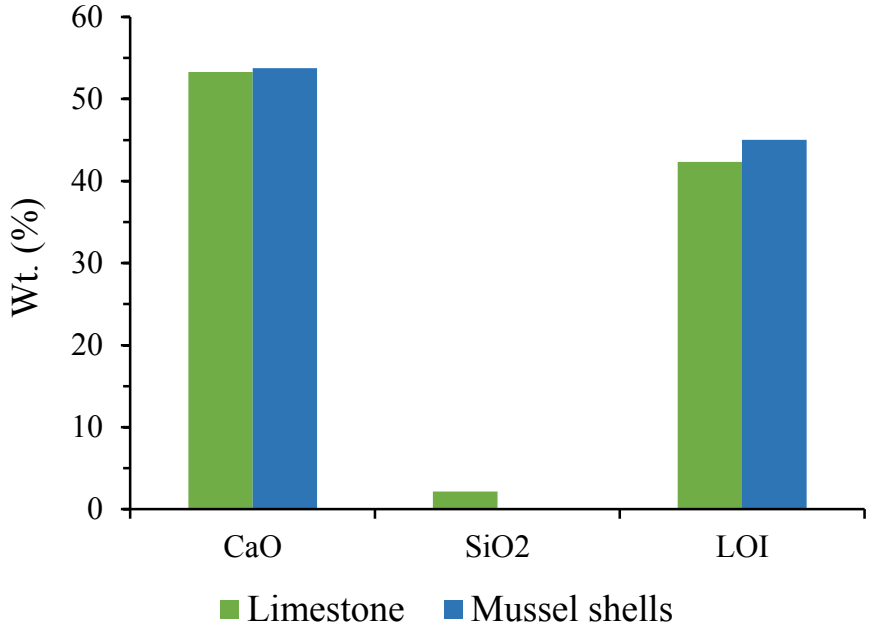


Figure 3-7. X-ray fluorescence: Major oxides in alkaline materials (average results from duplicate analyses).

For both mussel shell and limestone the main component was calcium (53.03 - 53.81 wt. % as CaO, respectively). The calcium carbonate ($CaCO_3$) content can then be approximated using either a conversion factor of 1.7846 (based on the molecular weight of each element in CaO and $CaCO_3$) or the loss on ignition values (LOI). Mussel shell and limestone had 98.8 and 95.6 wt. % $CaCO_3$ using the conversion factor, and 95.9 and 95.1 wt. % $CaCO_3$ using the CaO and the LOI values, respectively. As the purity of the alkaline materials has important repercussions on the alkalinity production, and therefore on the life-span of a system, a purity of > 90 % is usually recommended (ITRC 2012; Watzlaf et al. 2004). These results indicated

that both alkaline materials were very pure and therefore suitable for their use in SRBR, with the mussel shells potentially affording slightly more calcium carbonate than the limestone.

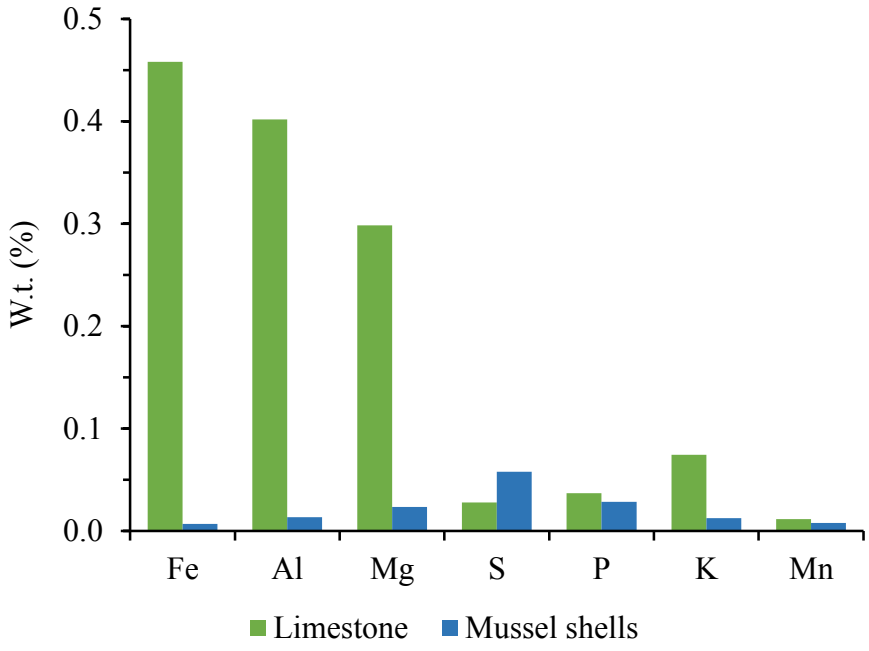


Figure 3-8. X-ray fluorescence: Trace elements in alkaline materials (average results from duplicate analyses).

Figure 3-8 shows that all other elements were found at trace level only (< 1 wt. %). Additionally, the XRF analyses confirmed the results from the hot acid digestions in showing that limestone contained more trace metals (especially Fe and Al) than the mussel shell. According to the XRF results, Fe and Al contents in limestone were respectively 65.4 and 28.7 times higher than in mussel shell (compared to only 5.5 and 3.5 times higher according to the hot acid digestions). While similar contents of Mn (0.008 and 0.012 wt. %) and Cu (0.004 and 0.003 wt. %) were measured in mussel shell and limestone respectively, neither Ni nor Zn was

detected at measurable levels (detection limit 0.001 wt. %). In contrast, mussel shells displayed twice the amount of S compared to the limestone. Although the difference is relatively small, the phosphorous content was unexpectedly higher in limestone compared to mussel shell with 0.037 and 0.029 wt. %, respectively.

Table 3-10 shows the results of the X-ray diffraction (XRD) analyses performed on the alkaline materials. Figure 3-9 and Figure 3-10 show the X-ray diffraction patterns for mussel shell and limestone, respectively.

Table 3-10. X-ray diffraction results (%) on alkaline materials (average results from duplicate analyses).

Phase name	Phase formula	Mussel shell	Limestone
Calcite	$CaCO_3$	4 %	99 %
Aragonite	$CaCO_3$	96 %	-
Quartz	SiO_2	-	1 %

While aragonite was the main mineral phase in the mussel shell followed by traces of calcite, only calcite was identified in the limestone pieces, along with traces of silicates (e.g. quartz, SiO_2). These findings will undoubtedly have important repercussions on the alkalinity generation and will be discussed in more details in Chapter 4.

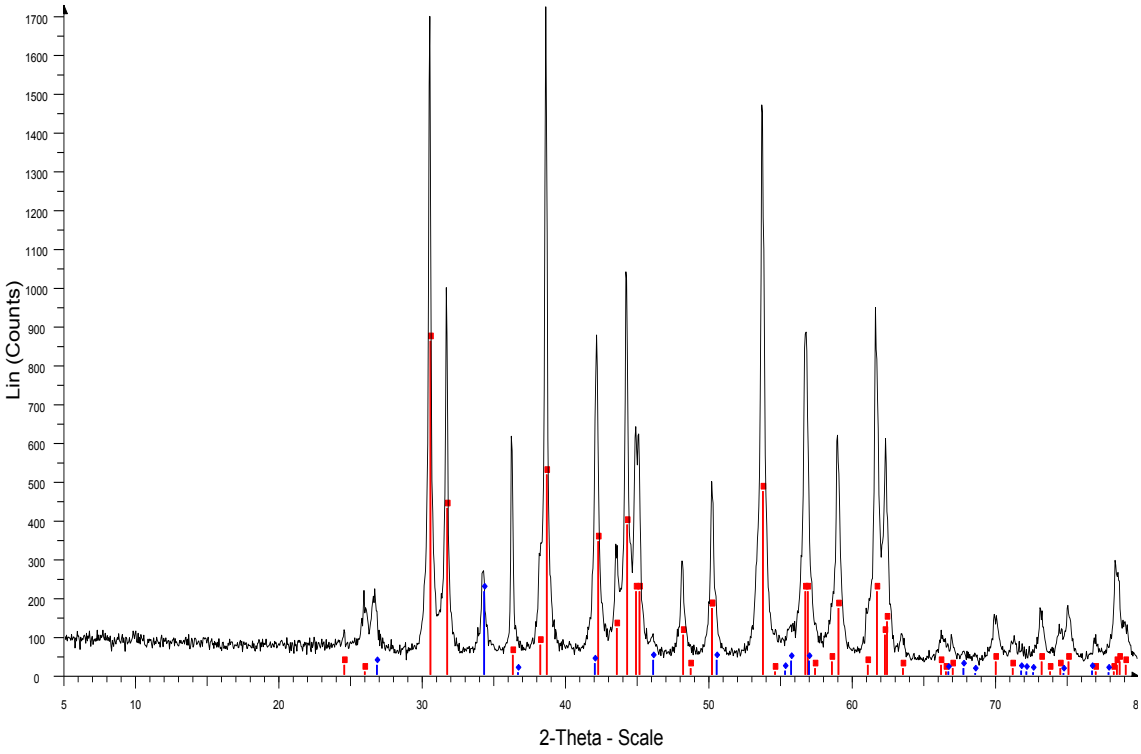


Figure 3-9. X-ray diffraction pattern of mussel shell. Red peaks indicate aragonite, blue peaks indicate calcite.

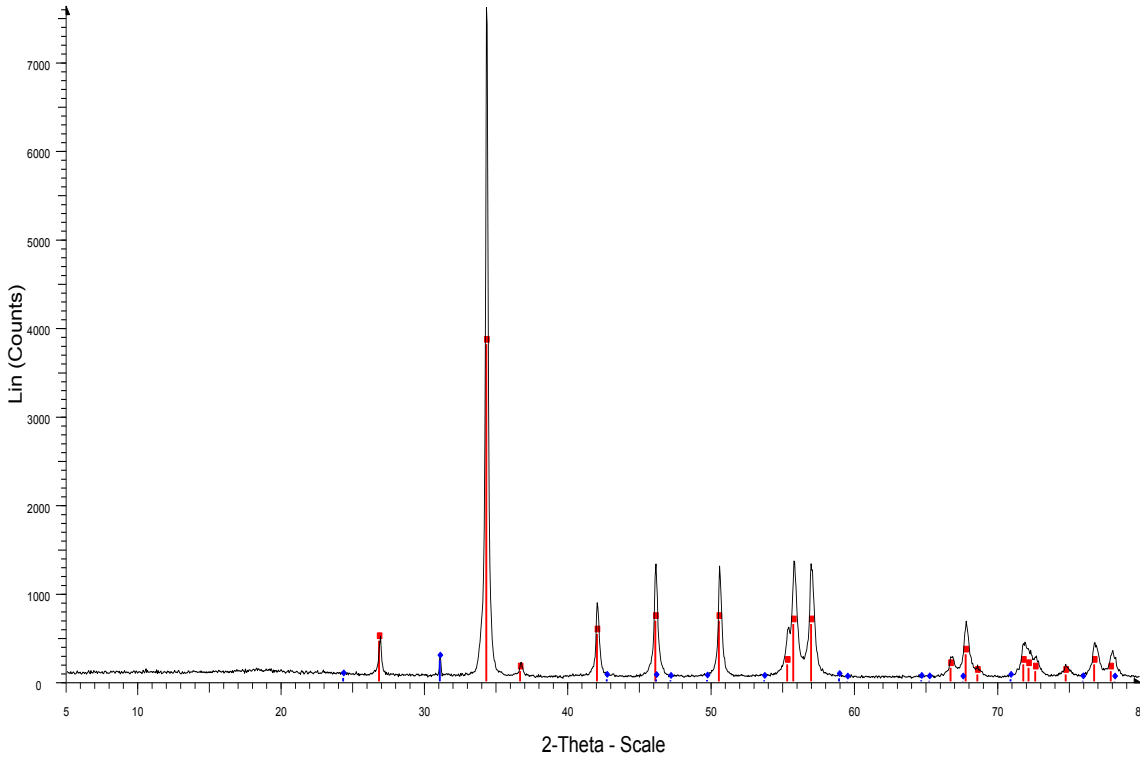


Figure 3-10. X-ray diffraction pattern of limestone. Red peaks indicate calcite, blue peaks indicate quartz.

3.5 Conclusions

The hydraulic parameters measured in this study confirmed that the risk of clogging and/or short-circuiting was low. The saturated hydraulic conductivities (6.29 x 10^{-2}, 9.72 x 10^{-2} cm/sec for the mussel and the limestone containing mixtures, respectively) were found to be well within the recommended range found in the literature, and at least three orders of magnitude higher than values measured in early studies where scientists and engineers used solely compost in the reactive substrates. The porosity was also found to be within the recommended range and indicated that approximately half of the total volume was occupied by voids and therefore available for the water to flow-through. The final HRT_n were computed at 3 and 10 days for the short and long HRT_n systems, respectively. Although, one could argue that the top and bottom gravel layers should not be included in the calculation of the total HRT_n (i.e. they are essentially non-reactive layers), it was estimated that due to the semi-continuous upward-flow approach (i.e. short pumping periods alternated with longer resting periods), partially treated MIW would occupy the gravel layers for long periods of time during which some sort of metal removal (e.g. sedimentation and (oxy)hydroxide precipitation mainly) would occur, and thus the whole volume should be taken into account. This was confirmed by the reactors autopsies (Chapter 5).

The chemical analyses showed that the organic materials were unlikely to contribute to high concentration of metals in the treated effluent. Indeed, only the compost material (used as a microbial inoculum and as a source of EAS important for the start-up period) was expected to completely degrade during the SRBR operational time period, and contained high concentrations of Fe, Al, Mn and Zn. In addition, the metal contents were found to be up to 7

times higher in the limestone compared to the mussel shell (based on the hot acid digestion results). This finding may have implications on the relative performances of mussel shell or limestone containing SRBR. Finally, the C/N ratios measured on the individual materials were potentially too high (N deficiency) to properly stimulate SRB growth and substrate biodegradability.

The mineralogical analyses indicated that both alkaline materials had a very high purity (≥ 95 wt. % $CaCO_3$). Of particular importance was the fact that the $CaCO_3$ in the mussel shell was composed of a mixture of mainly aragonite with traces of calcite, while in the limestone it was made of pure calcite. This finding will have important repercussions on the alkalinity generation as discussed later in Chapter 4. Finally, a theoretical approach suggested that the sulfate reduction pathway (i.e. the sulfidogenesis) would contribute to approximately 70 to 80 % of the total alkalinity generation in the bioreactors, the remainder attributed to the calcium carbonate dissolution. This hypothesis will be further examined in Chapter 4 along with data collected during the treatability study.

CHAPTER 4

MINE-INFLUENCED WATER TREATABILITY STUDY

4.1 Introduction

The objective of this study was to evaluate the effectiveness of four different mesocosms-scale SRBR to treat actual acidic mine-influenced water. The four treatment systems (operated in duplicate) tested two different hydraulic retention times (approx. 3 and 10 days) and two alkalinity amendment materials (limestone and mussel shell). The four systems were: mussel-shell short HRT (MS-S), mussel-shell long HRT (MS-L), limestone short HRT (LS-S), and limestone long HRT (LS-L). The complete experimental design was described previously in Chapter 3. The main focus of the present chapter was to evaluate the efficiency of the four treatment systems over a total period of 12 months (two 5-month periods of flow-through treatment separated by a 2-month interruption period). Of particular interest were the removal of acidity, metals and sulfate from the MIW. Influent, pore-water, and effluent samples were collected weekly for the first 5-month period and fortnightly during the second 5-month period, and analyzed for *in-situ* parameters including: pH, oxido-reduction potential (ORP), conductivity, temperature, and dissolved oxygen. In addition, the following parameters were also monitored: metal concentrations (total and dissolved), alkalinity (only measured in effluent samples), acidity (only measured in influent samples), sulfate and dissolved sulfide concentrations, nitrates, phosphates, Chemical Oxygen Demand, Total and Dissolved Organic Carbon. Unless stated differently, all analyses were conducted in the Environmental Laboratory of the Civil and Natural Resources Engineering Department at the University of

Canterbury. This chapter presents the results from the above-mentioned analyses and discusses the changes in water chemistry occurring within the SRBR systems.

4.2 Material and Methods

4.2.1 In-situ chemical analyses

All in-situ parameters (pH, ORP, conductivity, temperature and D.O.) were measured immediately upon collection using portable instruments and the data recorded when readings stabilized. Approximately 500 ml of sample were collected in PP bottles and the instrument probes inserted directly into the plastic bottles. All instruments were calibrated and/or checked weekly prior to sample collection. The pH meter (EDT RE-357Tx) was calibrated with pH 4.00 and, 7.00 standard buffer solutions and checked with a pH 10.00 standard solution. The conductivity and temperature meter (Thermo Orion 130A) was calibrated with a 0.01 M potassium chloride (KCl) solution (1413 µS/cm at 25°C). The D.O. meter was calibrated with oxygen-saturated water approximately once a month and checked prior to sample collection using the instrument's calibration chamber maintained at 100% water saturated air (note that D.O. was measured only during the second 5-month treatment period). The ORP-probe (YSI pH100) was checked in the laboratory approximately once a month using saturated solutions of quinhydrone at pH 4 and 7.

4.2.2 Alkalinity and acidity

Alkalinity was measured in effluent samples and acidity in the influent MIW according to Standard Methods 2320B and 2310B, respectively (APHA 2005). Alkalinity titrations were

performed using 0.1 M HCl to an endpoint of pH 4.5. Prior to measuring the acidity, a hot peroxide pretreatment was performed to oxidize all ferrous iron (Fe^{2+}) into ferric iron (Fe^{3+}) by adding 3 to 5 drops of 30 vol. % hydrogen peroxide (H_2O_2) and boiling the samples in a covered Erlenmeyer flask for about 3 minutes. The titration was performed using either 0.02 M or 0.1 M NaOH to pH 3.7 (mineral acidity) and pH 8.3 (total acidity). The pH meter (EDS RE-357Tx) was calibrated prior to measuring alkalinity and acidity with standard buffer solutions of pH 4.00 and 7.00 and checked with a pH 10.00 buffer solution. Both alkalinity and acidity are reported as mg/L of $CaCO_3$ equivalent according to Eq. 4.1.

$$\text{Alkalinity, Acidity (mg/L } CaCO_3 \text{ equivalent)} = (A \times N \times 50{,}000) / \text{mL of sample} \quad (4.1)$$

Where:

A = mL of standard acid/base used,

N = normality of the acid/base used.

4.2.3 Metals and cations

Samples for total and dissolved metals analyses were collected in separate 120 mL PP bottles, preserved with concentrated nitric acid (69%, Fisher trace analysis grade) to reduce pH < 2, and stored at 4°C until analysis via inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500cx) according to Standard Method 3125B (APHA 2005). Dissolved metals were filtered immediately upon collection through a 0.45 µm syringe nylon filter, and total metals were obtained by digestion following the method established by Wicke et al. (2012). Briefly,

the samples were thoroughly mixed and 25 mL aliquots were transferred into a 50 mL centrifuge tube. After the addition of 5 mL of concentrated nitric acid (69%, Fisher trace analysis grade), the closed centrifuge tubes were placed into a heating block and boiled for 1 h. Cooled samples were then filtered (0.45 μm) and analyzed via ICP-MS. Quality assurance and control (QA/QC) was achieved through the use of accredited standards, blanks, and duplicate analyses (10%)[3]. The metals measured were Al, Fe, Mn, Ni, Cu, Zn, Cd, Pb, Cr, Co as well as the cations Ca, Mg, Na and K.

Ferrous iron (Fe^{2+}) concentrations were measured within 24 h on non-filtered samples using a HACH spectrophotometer (DR-2500, HACH instrument method 8146). Samples were diluted to 10 or 25 ml in instrument-specific glass cuvettes. Detection limit was 0.02 mg/L. Ferric iron (Fe^{3+}) was later calculated by difference between total Fe and Fe^{2+}.

4.2.4 Sulfate and sulfide

Sulfate (SO_4^{2-}) samples were filtered at 0.22 μm upon collection and stored at 4°C until analysis using a Dionex ion chromatograph (Dionex ICS-2000) at the Geology Department at the University of Canterbury. Again, QA/QC was achieved through the use of accredited standards, blanks, and replicate analyses.

[3] 10 % means that one blank and one replicate sample were analyzed every ten samples. In addition to the National Institute of Standards and Technology (NIST) standard used for instrument calibration and recovery checks, one spiked samples was analyzed every 20 samples as a specific in-run recovery check (using Rh).

Dissolved sulfide (S^{2-}) concentrations were analyzed immediately upon collection using a HACH spectrophotometer (DR-2500, HACH method 8131). Unfiltered samples were diluted to 25 ml in instrument-specific glass sample cells. Detection limit was 5 µg/L.

4.2.5 Carbon content

Chemical Oxygen Demand (COD) was analyzed within 24 h on a HACH spectrophotometer (DR-2000, Environmental laboratory specific method). COD is commonly used to indirectly quantify the amount of organic compounds present in a water sample. Total and Dissolved Organic Carbon (TOC, DOC) were analyzed at the Chemical and Process Engineering Department at the University of Canterbury using a Shimazo TOC Analyzer. Samples for DOC measurements were filtered at 0.45 µm upon collection, preserved with 0.2 M sulfuric acid (H_2SO_4) and stored at 4°C until analysis. Standard solutions of known concentration of total carbon (TC, 1000 mg/L) and inorganic carbon (IC, 1000 mg/L) were used for calibration and quality control purposes. Standard solutions were prepared by dissolving reagent grade potassium hydrogen phthalate (TC) and sodium hydrogen carbonate and sodium carbonate (IC). Note that COD was measured only during the first 5-month period (week 1 to 20), while TOC and DOC were analyzed only during the second 5-month period (week 22 to 40)[4].

[4] The Civil and Natural Resources Engineering Department did not have a TOC analyzer by the time the treatability study started in March 2013. A TOC analyzer was purchased by the Chemical and Process Engineering Department in July 2013 and enabled this measurement to be initiated.

4.2.6 Nutrients

Nitrates (NO_3^-) and phosphates (PO_4^{3-}) were analyzed upon collection using a HACH spectrophotometer (DR-2500, HACH method 8039 and 8114, respectively). Samples were diluted to 10 or 25 ml in instrument-specific glass sample cells. Both NO_3^- and PO_4^{3-} were analyzed in influent, pore-water and effluent samples from week 22 onwards (second 5-month period only). Detection limits were 0.3 mg/L for both nitrate and phosphate.

4.2.7 Statistical analyses

Statistical analyses were performed with R Statistics version 3.2.0 (CoreTeam 2013). Statistical differences between the four treatment systems (MS-S, MS-L, LS-S, LS-L) were analyzed by two-way ANOVA analyses. For each individual parameter (e.g. pH, ORP, metal concentrations, etc.), entire data sets from the weekly or fortnightly analyses were used (n ranged between 10 and 30).

4.2.8 Geochemical modeling

The chemical speciation model PHREEQC version 3.0.6 (Appelo and Parkhurst 1999) with the MINTEQV4 database was used to calculate metal speciation and saturation indices (SI) of selected mineral phases. Effluent or pore-water parameters were used in the model. Further modeling was performed later and presented in Chapter 5 after the presentation of the spent substrate analyses (Geochemical modeling section 5.3.4).

4.2.9 New Zealand water quality guidelines

Water quality guidelines published by the Australian and New Zealand Environmental and Conservation Council (ANZECC 2000) were used to evaluate the severity of the MIW by comparing in-stream metal concentrations with the environmental trigger values (TVs) presented in both the Guidelines for Freshwater Aquatic Ecosystems and the Guidelines for Recreational Purposes. The Guidelines for Freshwater Aquatic Ecosystems propose separate TVs for different levels of protection (80%, 90%, 95% and 99%). The TVs are based on international ecotoxicity studies and indicate the percentage of aquatic species expected to be unaffected by the contaminated waters. As mining sites are highly disturbed environments, the 80% level of protection was the most applicable. However, because of insufficient data, there were no proposed TVs for Fe and Al at pH < 6.5 in the Guidelines for Freshwater Aquatic Ecosystems and therefore the Guidelines for Recreation Purposes have been used instead. These guidelines' primary objective is the protection of human health, mainly avoiding skin irritation and sickness upon ingestion of 100 mL of polluted water. Table 4-1 shows the TVs from the Guidelines for Freshwater Aquatic Ecosystem and the Guidelines for Recreational Purpose. Recommended maximum sulfate concentrations and recommended pH range are also indicated in Table 4-1.

Table 4-1. Trigger values from the Guidelines for Freshwater Aquatic Ecosystems and the Guidelines for Recreational Purposes (ANZECC 2000) for dissolved metal concentrations, sulfates and pH (metal and sulfate concentrations are in mg/L).

Water parameters	Guidelines for Freshwater Aquatic Ecosystems (80% protection level)	Guidelines for Recreational Purposes
Fe	ND	0.30
Al	ND	0.20
Mn	3.60	0.10
Zn	0.03	5.00
Cu	0.0025	1.00
Ni	0.0017	0.10
Cd	0.0008	0.005
Sulfate	ND	400
pH	ND	6.5 - 8.5

ND = not defined

4.3 Results and Discussion

4.3.1 Mine-influenced water influent quality

The MIW was sourced in 1 m^3 batches, approximately every six weeks, at an active coal mine situated on the West Coast of the South Island of New Zealand. In total, 5 batches were collected between January and December 2013. The first batch of MIW was collected in a stream flowing downstream of the mine site and represented MIW diluted with un-impacted surface water (the collection point was located approximately 500 meters below the main waste dumps), and the remaining four batches were collected in a sedimentation pond situated at the bottom of the main waste rock dumps (Figure 4-1). Table 4-2 shows the influent median values of in-situ parameters, metal and sulfate concentrations, and acidity of each batch of MIW used during the treatability study. Additionally, conductivity, pH, ORP and temperature were also measured on-site, during MIW collection, using pre-calibrated portable instruments. One supplementary sample, collected with batch #1, was analyzed for metal concentrations immediately upon return to the University of Canterbury. The in-situ parameters as measured upon collection at the mine site and the metal concentrations measured in the supplementary sample collected with batch #1 are also shown in Table 4-2.

Because TVs are only applicable to concentrations of contaminants in flowing streams where dilution and mixing is occurring, the metal concentrations in MIW batches #2, #3, #4, and #5 (collected from the sedimentation pond) cannot be compared directly to the TVs, and only the first batch of MIW (collected from a flowing stream) can be compared to the TVs. When comparing the metal concentrations measured in batch #1-supplementary sample with the TVs

(Table 4-1 and Table 4-2), seven metals of concern were identified: Fe, Al, Mn, Zn, Cu, Ni and Cd. Measured metal concentrations ranged from 2.5 (Cd) to 247 times (Ni) the TVs.

Figure 4-1. (A) In-stream mixed MIW downstream of mine site where batch #1 was collected; (B) Sedimentation pond where batches #2 to #5 were collected (the waste dumps can be seen at the back of picture B).

Table 4-2. Geochemical signatures of each batch of influent MIW used during the treatability study, and supplementary sample collected with batch #1. Median values of in-situ parameters, dissolved metals and sulfate concentrations (mg/L), and total acidity (mg/L $CaCO_3$ equivalent). Values in brackets indicate on-site measurements.

Water quality parameters	Suppl. Sample (with #1)	MIW batches				
		#1	#2	#3	#4	#5
pH	3.58	4.32 (3.58)	2.92 (2.65)	2.89 (2.66)	2.82 (2.94)	2.85 (2.69)
Conductivity (µS/cm)	1327	1239 (1327)	2360 (2077)	2480 (2210)	2385 (1674)	2605 (1998)
ORP (mV)	328	266 (328)	456 (473)	469 (461)	481 (476)	468 (468)
Temperature (°C)	13.1	18.8 (13.1)	15.5 (16.2)	15.0 (9.8)	18.2 (10.3)	19 (12.3)
Dissolved oxygen (%)	n.d.	n.d.	n.d.	n.d.	95.2	96.4
Sulfate	810	810	1570	1813	1526	1655
Total acidity	n.d.	n.d.	250	460	360	340
Fe (total)	10.41	0.2	12.22	48.84	27.19	15.86
Fe^{2+}	n.d.	n.d.	0.7	1.54	0.93	0.76
Fe^{3+}	n.d.	n.d.	11.52	47.3	26.26	15.10
Al	4.66	2.02	13.32	18.88	14.81	15.48
Mn	9.61	4.78	13.78	18.13	15.41	16.17
Zn	1.37	0.64	3.63	4.94	3.92	4.51
Cu	0.033	0.024	0.31	0.17	0.14	0.11
Ni	0.42	0.22	0.95	1.14	0.88	1.00
Cd	0.002	0.0004	0.003	0.003	0.002	0.003
Collection date (2013)	12th Jan	12th Jan	18th Apr	13th Jun	9th Oct	17th Dec
Period of usage in SRBR (weeks)	n.a.	1 to 5	6 to 13	14 to 20	21 to 29	30 to 41

n.d. = not determined; n.a. not applicable

The MIW in the laboratory was continuously aerated with an air pump before being conveyed into the SRBR in order to simulate the aerobic and oxidizing conditions measured at the mine site. By comparing the values measured at the mine site with the median values measured in the laboratory (see pH, conductivity, ORP and temperature values in brackets vs. not in brackets in Table 4-2), it was noted that, apart from batch #1 for which the in-situ parameters did changed substantially, the measured values for the other 4 batches did not vary greatly between the collection site and the laboratory; and the following differences were observed: pH values generally increased by < 0.3 pH units, conductivity increased by 270 (batch #3) to 711 µS/cm (batch #4), and ORP varied by < 5 %. Temperature was higher in the laboratory compared to the collection site, but little variation (< 5°C) was observed over the 41-week treatment period. Overall, batch #3 had the larger concentrations of Fe, Al, Mn, and sulfate and represented the most severe MIW used in the present study. Influent samples were taken from a feeding pipe after the peristaltic pumps and before the MIW entered the SRBR (no samples were taken directly from the MIW feeding tank). As stated in Chapter 3, the inflow was maintained at 4.5 L/d for the short HRT and at 1.5 L/d for the long HRT.

4.3.2 Effluent and pore-water quality

Effluent, pore-water, and influent samples were collected weekly for the first 5-month period of treatment and fortnightly for the second 5-month period. Several parameters including alkalinity, sulfate, sulfide and metal removal fluctuated during the first 7 to 8 weeks of MIW treatment. These fluctuations are common in new waterlogged systems as the biogeochemistry establishes (Dvorak et al. 1992; Wildeman et al. 2006). On the other hand, the in-situ parameters showed steady-state values from week one onward. Additionally, because the first

batch of in-stream mixed MIW was considered to have too weak a typical MIW signature to accurately reflect SRBR operational capacity, the results of the first 5 weeks of treatment (when batch #1 was in use) are not considered in the following sections of this chapter. Appendix A-1 shows the raw data of all water quality parameters measured during the 41 weeks of treatment.

4.3.2.1 In-situ parameters

Figure 4-2 shows the changes in pH, ORP, and conductivity throughout the 10-month flow-through treatability experiment (note that the D.O. concentration results for the second 5-month treatment period is only reported in Appendix A-1; because D.O. measurements by probe can produce erroneous values due to rapid re-aeration of the samples it was decided that the D.O. values were not reliable, and the subsequent analysis of results is based on the ORP measurements instead). Median pH increased from 2.89 in the influent MIW to effluent values of 6.43 (MS-S), 6.73 (MS-L), 6.14 (LS-S), and 6.36 (LS-L). Pore-water pH differed by ≤ 6 % from the effluent pH, indicating that the bulk of the neutralization occurred in the lower half of the reactor (i.e. beneath the pore-water effluent port). Pore-water median pH values (6.09 MS-S, 6.74 MS-L, 6.21 LS-S, 6.45 LS-L) were generally slightly higher than the effluent pH values (apart from system MS-S). This observation could be explained by two reasons: (1) effluent samples became in contact with atmospheric oxidizing conditions and CO_2 degassing would have slightly decreased their pH; and (2), as mentioned earlier in Chapter 3, oxygen sometimes entered the systems through the pore-water sampling ports during sampling events, which could have led to the oxidation (and subsequent hydrolysis) of dissolved divalent metals present in the higher (second) half of the reactors.

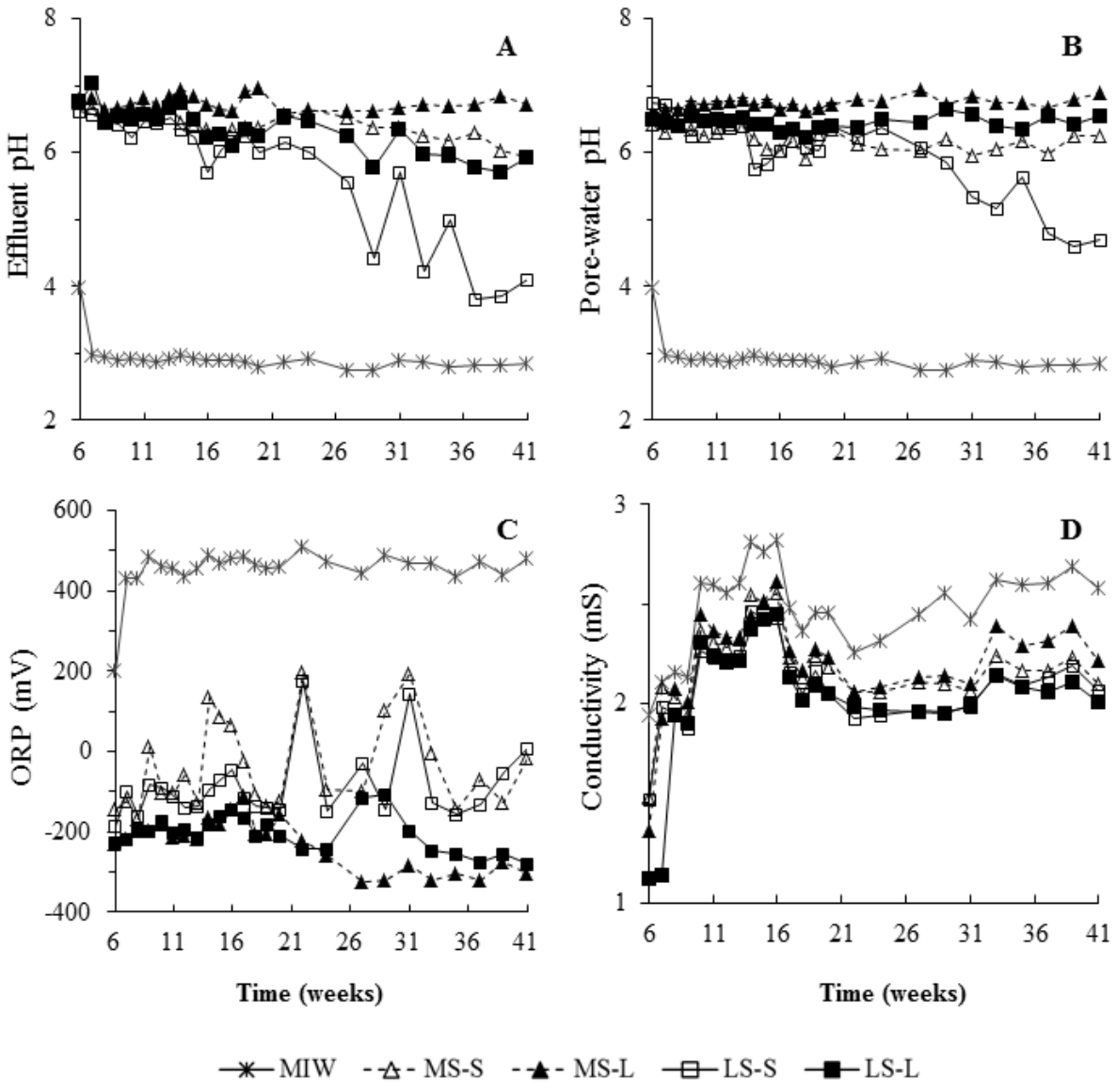


Figure 4-2. Changes in in-situ parameters over time: (A) effluent pH, (B) pore-water pH, (C) pore-water ORP, (D) effluent conductivity.

Overall, reactors operating at a longer HRT and/or containing mussel shells showed a higher effluent and pore-water pH compared to reactors operating at a shorter HRT and/or containing limestone (Figure 4-2 A and B). Statistical tests showed that pH differences between short and long HRT, as well as between mussel shell and limestone systems, were both statistically very significant ($p < 0.001$). From week 1 to 20 (first 5-month treatment period), the four systems resulted only in small differences in effluent pH, while during the second part of the treatability study (week 21 to 41), the disparities in effluent pH between each system were more noticeable. These differences were also more pronounced in the effluent samples than in the pore-water samples. While system LS-L performed better in terms of raising pH than system MS-S during the first treatment period, the opposite was true during the second treatment period (valid for effluent pH only, not for pore-water pH), suggesting that the acidity mitigation in the higher (second) half of the limestone reactors was somehow diminished. Figure 4-2 (A, B) also shows that system LS-S performance started to decline after week 24 (effluent values) and after week 27 (pore-water values) until the end of the treatability experiment again indicating that the neutralization processes (sulfate reduction and/or $CaCO_3$ dissolution) were becoming less effective.

Figure 4-2 (C) shows ORP changes in the pore-water samples (ORP values in effluent samples underwent rapid changes once the treated water exited the reactors and started to oxidize and were therefore less representative of the actual conditions within the reactors). Overall, all four systems considerably reduced the ORP from an influent median value of 465 mV to median pore-water values of -70 mV (MS-S), -216 mV (MS-L), -115 mV (LS-S) and -199 mV (LS-L). The longer HRT systems resulted in lower ORP values and a statistical test confirmed that

the HRT had a very significant influence ($p < 0.001$) on the ORP. On the other hand, the use of mussel shell instead of limestone did not result in a significant difference ($p = 0.36$). Until week 20, ORP values in the pore-water samples were consistently reducing (negative ORP values) except for system MS-S which showed positive values during four sampling events (out of 15 sampling events). Three of these four events happened consecutively when MIW batch #3 was in use (week 14 to 20). This indicated that the microbial community may have been impacted by the higher metal and acidity loading (since treatment efficiency in terms of pH, alkalinity generation and metal removal also declined during these three weeks). In addition, it worth noting that reactor MS-S encountered more frequent issues associated with oxygen ingress through the pore-water sample port, likely increasing the ORP values. Both long HRT systems constantly had negative ORP values (LS-L stayed under -100 mV and MS-L stayed under -200 mV over the entire 41 weeks of treatment). As SRB are thought to require an ORP of at least -100 mV, and because the lower the ORP, the higher their microbial activity should be (Postgate 1979; Zagury et al. 2006), the strongly negative values reported for the reactors operating at longer HRT suggested that these systems had more active sulfate-reducing bacterial communities. This last observation was later confirmed by both the sulfate and metal removal efficiencies (Figure 4-8 A,Figure 4-10, and Figure 4-11) which were higher in the long HRT systems, but not by the sulfide measurements which did not show any significant difference between the short and long HRT (Figure 4-8 B).

Figure 4-2 (D) shows how the conductivity changed through the experiment. Compared to the pH and the OPR values, the influent conductivity fluctuated more over time, reflecting both changes in the MIW chemistry on the mine site (e.g. changes in ion concentrations in the MIW

due to more or less weathering of the waste dumps) and possible changes occurring in the feeding tank (e.g. metal hydrolysis). Effluent values were constantly lower than the influent values suggesting that MIW treatment was efficiently removing ions (i.e. metallic cations and sulfate) from influent MIW. Statistical analyses indicated that the alkalinity source had a significant influence on the conductivity ($p < 0.01$) with the mussel shell systems resulting in a higher conductivity than the limestone systems. This was attributed to a faster dissolution of the mussel shells compared to the limestone (therefore releasing more ions in solution). The HRT had no significant influence on the effluent conductivity ($p = 0.776$). Changes in temperature were minimal between the SRBR's effluent and influent MIW and no difference in temperature was observed within the four treatment systems (Appendix A-1).

4.3.2.2 Nutrients and organic carbon analyses

Figure 4-3 shows the nutrient (nitrate and phosphate) concentrations in the influent, effluent and pore-water samples from week 22 to 41.

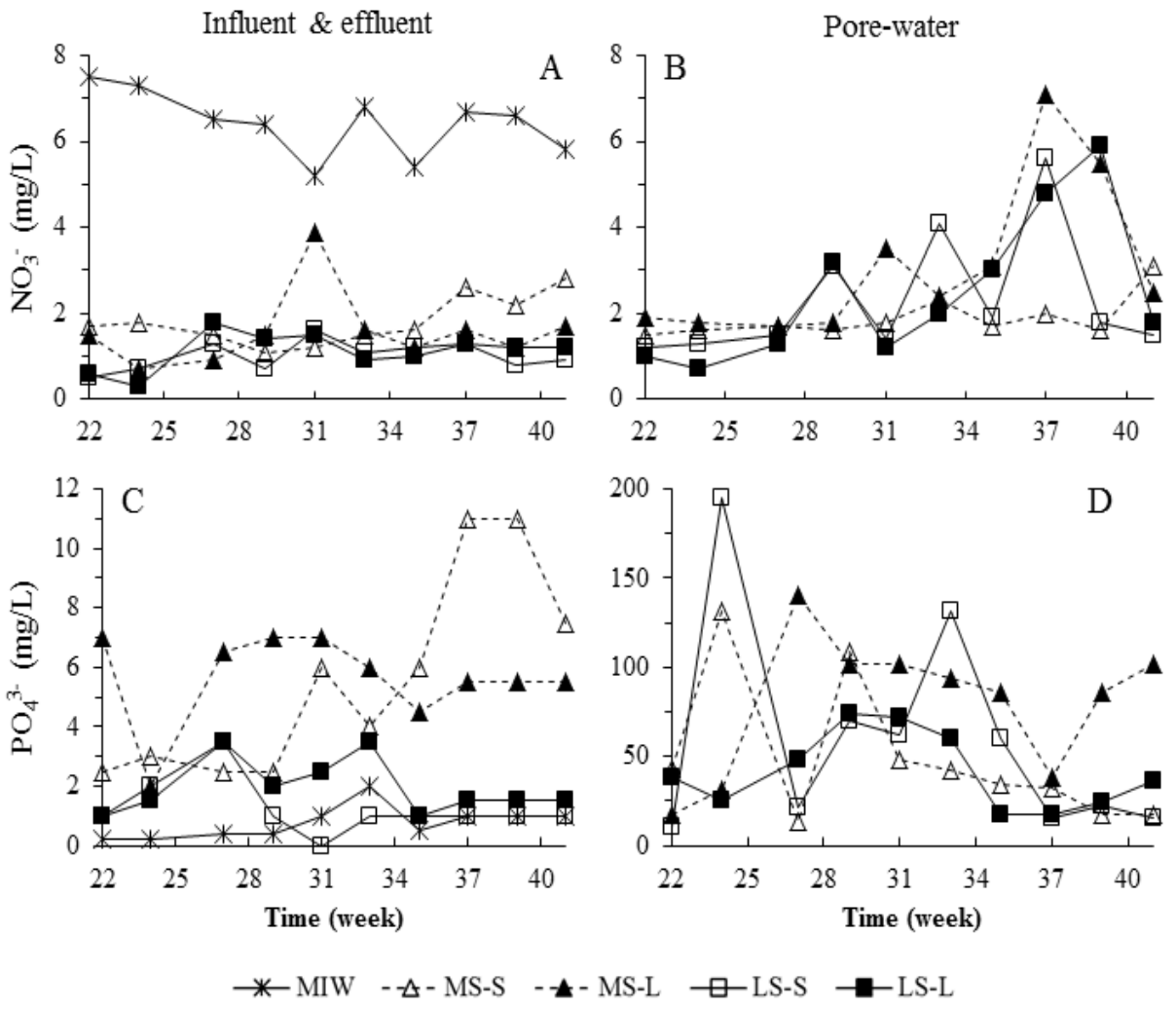


Figure 4-3. Nutrient concentrations (mg/L) over time: (A) Nitrate in influent and effluent samples; (B) Nitrate in pore-water samples; (C) Phosphate in influent and effluent samples; (D) Phosphate in pore-water samples.

Figure 4-3 (A and B) indicated that NO_3^- was consumed in the SRBR (i.e. the nitrate concentration was higher in influent MIW compared to pore-water or effluent values). Nitrate concentrations in uncontaminated water are typically low (< 5mg/L) and NO_3^- in MIW can originate from the weathering of the bedrock that contains nitrogen and from the use of ammonia-based explosives used during blasting (Bosman 2009). The consumption of nitrate within the SRBR was attributed to both biomass growth and denitrification. As the D.O.

decreases, denitrification becomes an important and necessary process in SRBR (i.e. because oxygen and nitrates are, in terms of energy available, more favorable electron acceptors than the sulfates, most of the oxygen and the nitrates must be consumed before the reducing conditions suitable for the sulfidogenesis are established, White and Gadd (1996)). A statistical test indicated that the alkalinity source significantly influenced the nitrate concentration in the effluent samples ($p < 0.01$), with the mussel shells resulting in a higher NO_3^- concentration. This result was attributed to the higher nitrogen content of the mussel shell compared to the limestone. No significant differences were observed between HRT.

By contrast, PO_4^{3-} was released from the substrate materials and then consumed within the SRBR (phosphate concentrations in influent MIW were much lower than pore-water concentrations, but only slightly lower than the effluent concentrations). The consumption of phosphate within the SRBR was attributed to biomass growth, and to the possible precipitation of metal phosphate minerals as discussed later in Chapter 5. Similarly to the nitrate results, systems containing mussel shell had a positive and very significant influence on the PO_4^{3-} concentrations in the effluent ($p < 0.001$), but the HRT had none. It is worth noting that the naturally colored effluents typical of SRBR may have interfered with the colorimetric tests and potentially resulted in nitrate and phosphate concentration higher than reality. Nevertheless, the relative concentrations and differences observed in the present study are thought to be representative of real changes occurring in between the four systems studied.

Figure 4-4 shows the organic carbon results (COD, TOC and DOC). Note that COD was measured only during the first 5-month treatment period and TOC and DOC only during the second 5-month period.

Similarly to the alkalinity data, the COD values largely fluctuated until reaching a steady-state behavior around week 8. Statistical results indicated that the HRT had a bigger influence than the alkalinity source (HRT had a significant influence on the effluent values with $p < 0.001$, and on the pore-water values with $p < 0.05$; the alkalinity source had none with $p = 0.830$ for effluent values, and $p = 0.094$ for pore-water values).

In contrast with the COD results, the TOC and DOC data showed a less clear influence of the HRT (Figure 4-4 C and D). Again this observation was supported by the statistical analyses indicating that the HRT had a significant influence ($p < 0.01$) on the DOC, but not on the TOC (the longer HRT resulted in higher concentrations of DOC), while the alkalinity source had a small influence on the TOC values only ($p < 0.05$, the mussel shell resulted in higher TOC values). Although these carbon analyses were not directly related to carbon substances readily usable by the SRB, these present observations suggested that the use of mussel shell did not result in significantly higher substrate degradation, nor in a higher release of organic carbon. On the other hand, the HRT appear to have influenced the carbon release, with the long HRT resulting in higher effluent carbon concentrations.

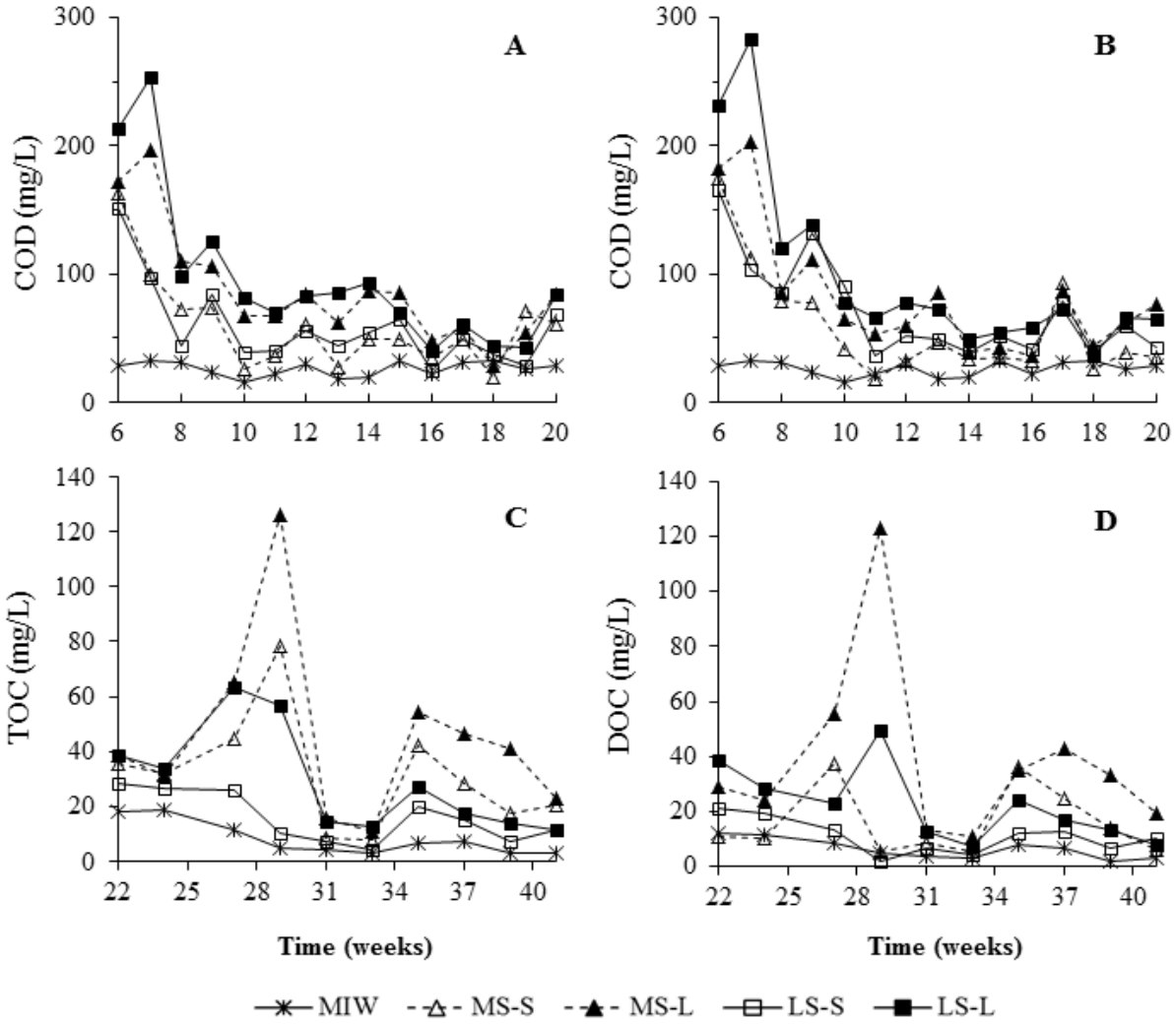


Figure 4-4. Carbon concentrations (mg/L): (A) influent and effluent COD, (B) influent and pore-water COD, (C) influent and effluent TOC, (D) influent and effluent DOC.

In a recent study, Trumm et al. (2014) successfully used SRBR filled with fresh waste mussel shells only (i.e. no organic materials were added to the substrate mixture) to treat MIW. The authors suggested that organic wastes still attached to the shells (e.g. seaweed and mussel meat remains) provided enough organic matter for the SRB to conduct sulfidogenesis. By contrast, the mussel shells used in the present study had been stockpiled outside for at least one year prior to their use in the SRBR, and were therefore in an advance stage of weathering. This

weathering would have washed out the more labile and easily available organic compounds and therefore, likely explained why the use of mussel shell did not result in a higher carbon release compared to the limestone. In addition, while a direct comparison between the COD and the TOC/DOC analyses is unpractical, the 2-month interruption period did not result in a noticeable increase of biodegradability of the substrate (i.e. the carbon release did not increased substantially after the 2 months of interruption).

4.3.2.3 Acidity and alkalinity

Figure 4-5 shows the mineral acidity (titration to pH 3.7) and the total acidity (titration to pH 8.3) measured in the influent MIW. Acidity stayed rather constant within each batch of MIW, but varied in between batches (batch #2 contained the lowest acidity, while batch #3 contained the highest acidity). These differences, similar to the variations seen in metal concentrations between batches, reflected the variability and changes occurring in MIW.

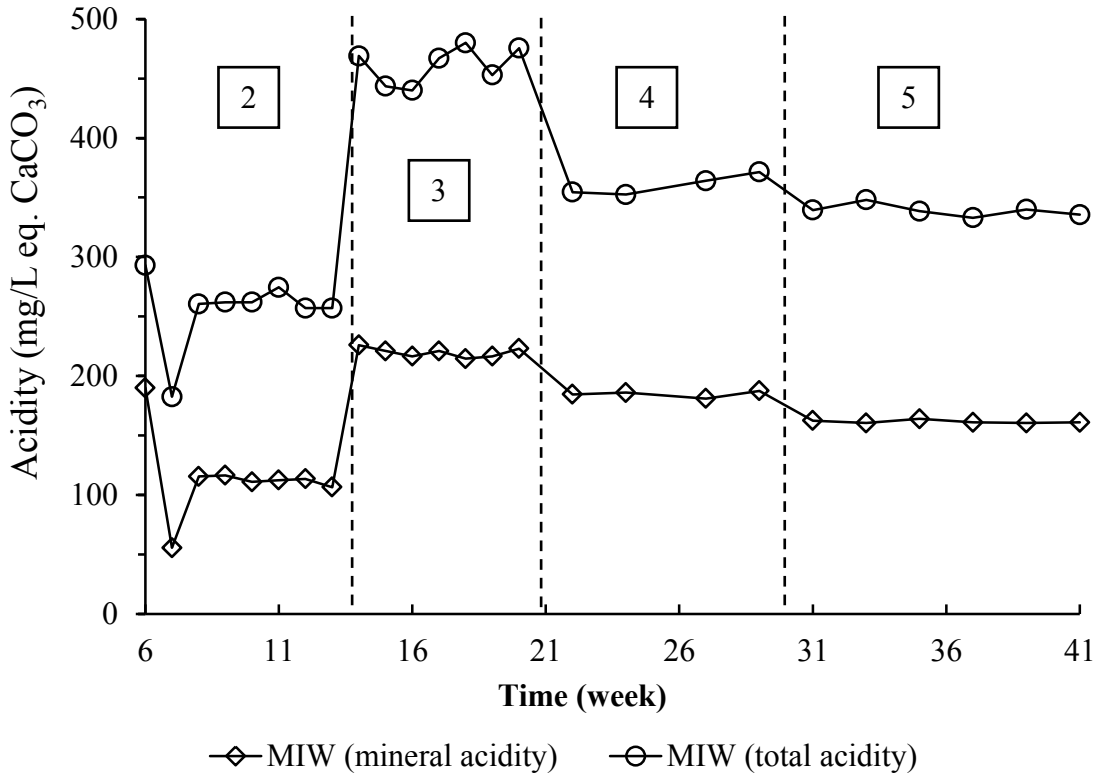


Figure 4-5. Mineral and total acidity (mg/L $CaCO_3$ eq.) measured in the influent MIW. Vertical dash lines indicate when a new batch of influent MIW was used, numbers indicate batch #.

Figure 4-6 shows the alkalinity in the effluent samples. As previously mentioned, the alkalinity fluctuated until it reached a steady-state behavior approximately at week 8 or 9. Table 4-3 shows median effluent alkalinity concentrations for each system for the following periods: week 8 to 20 (after stabilization until the end of the first treatment period), week 22 to 41 (after the 2 month interruption until the end of the experiment), and for the overall period of week 8 to 41.

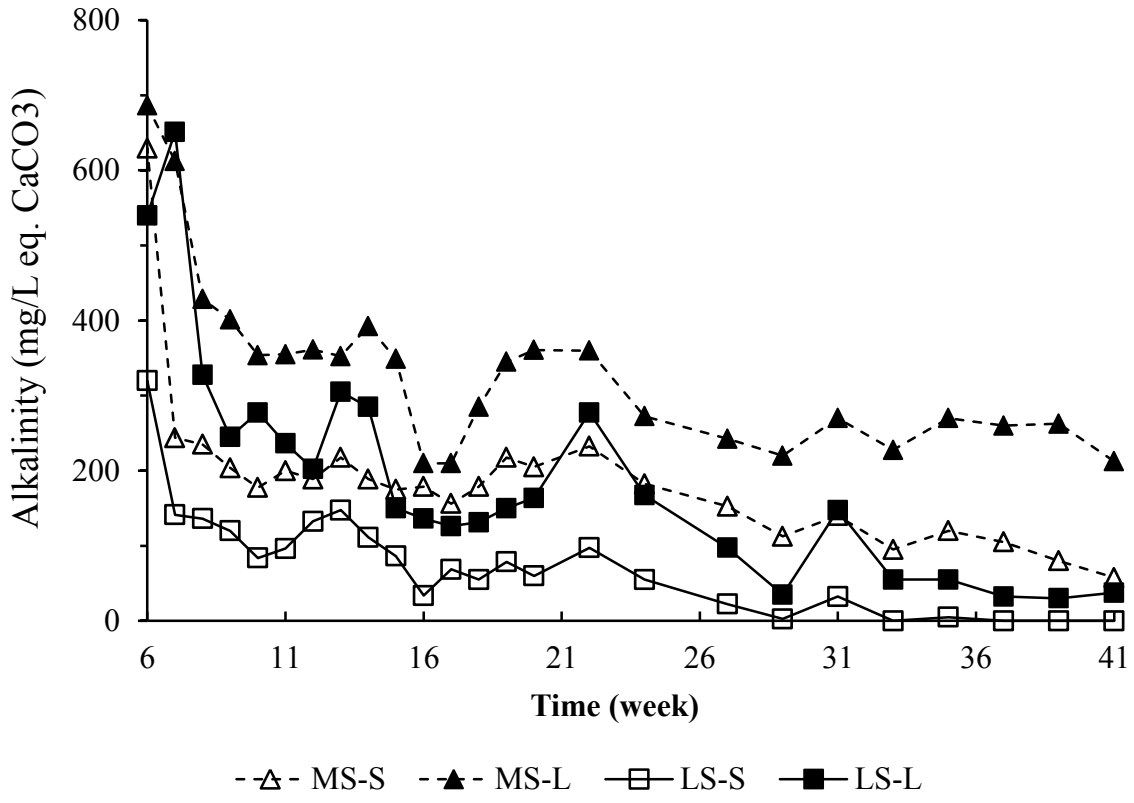


Figure 4-6. Effluent alkalinity (mg/L $CaCO_3$ eq.).

Table 4-3. Median effluent alkalinity (in mg/L $CaCO_3$ eq.).

	MS-S	MS-L	LS-S	LS-L
Week 8 - 20	188.8	353.8	86.3	202.5
Week 22 - 41	116.3	261.3	3.75	55.0
Week 8 - 41	178.8	285.0	60.0	150.0

Statistically very significant differences ($p < 0.001$) between MS-L and the three other systems confirmed that it was consistently the most effective reactor at generating alkalinity, followed by systems MS-S, LS-L and LS-S. Overall, treatment systems containing mussel shells significantly produced greater alkalinity ($p < 0.001$) than systems containing limestone at the same HRT, and systems operating at a longer HRT significantly produced more alkalinity ($p <$

0.001) than systems running at a short HRT. After the first five-month period of continuous operation (week 8-20) alkalinity generation from mussel shell and limestone was not exhausted, nor had it obviously diminished. But during the second treatment period (week 22-41), the systems containing limestone showed an important decrease in alkalinity generation. In addition, from week 15 onwards, system MS-S generally produced more alkalinity than system LS-L, indicating that the alkalinity generation was driven by its source rather than by the HRT.

The most probable reason for the greater alkalinity generation from the mussel shell was a faster dissolution rate. Different properties, including mineralogical phases, reactive surface area, grain size, and total calcium carbonate content, might explain this difference. As discussed in Chapter 3, the calcium carbonate present in the limestone was found to be purely calcite (approx. 99 wt. %), while mussel shells were comprised of a mixture of calcite (approx. 4 wt. %) and aragonite (approx. 96 wt. %). Cubillas et al (2005b) who examined the dissolution rates of various bivalves, including mussel shells, and compared them with the dissolution rates of both abiotic calcite and abiotic aragonite showed that (1) the geometric surface area-normalized dissolution rate of biotic calcite and aragonite corresponded to the dissolution rate of the abiotic minerals; (2) the geometric surface area-normalized dissolution rate of abiotic aragonite was approximately 30 % higher than the corresponding dissolution rate of abiotic calcite; and (3) the BET surface area of the mussel shells increased by 80 % during the dissolution experiment compared to other bivalves (e.g. clams and cockles which are also mostly comprised of biotic aragonite). In light of these results, it was clear that the mineral composition of the alkaline materials had a strong influence on the alkalinity

generation within the SRBR and that the presence of aragonite resulted in significantly higher alkalinity generation. Another study by Cubillas et al. (2005a), showed that the dissolution of aragonite was less affected by mineral armoring or coating than the dissolution of calcite. However, limestone passivation by coating of ferric iron precipitates is unlikely to happen in reducing systems like SRBR (Thomas and Romanek 2002a; URS 2003) and the inspection of limestone pieces post-treatment did not reveal any coating by iron (oxy)hydroxides (Figure 7-1, Appendix B). Furthermore, nitrogen present within the proteins in the periostracum (outer layer of the mussel shells) may have supported a greater microbial community and contributed to a higher alkalinity generation via SRB neutralization (Abdulkarim et al. 2013; McCauley et al. 2009). However, this last hypothesis was not supported by the sulfate measurements, as little and inconsistent differences in sulfate removal were observed between mussel shell and limestone systems (see section on sulfate removal below).

Figure 4-7 shows an estimate of the partitioning of alkalinity generation (for week 8 to 41) between that estimated from the calcium carbonate dissolution and estimated from the sulfate-reducing bacteria neutralization (i.e. sulfidogenesis). SRB contribution to total alkalinity generation was estimated using the influent and effluent sulfate concentrations along with Equations 2.11 and 2.12 (1 mole of sulfate reduced produced 2 moles of bicarbonate) and assuming that after the initial sorption onto the substrate materials, the only pathway of sulfate removal was through microbial reduction. $CaCO_3$ contribution to total alkalinity generation was obtained using the influent and effluent calcium concentrations along with Equation 2.19 (1 mole of calcium carbonate dissolved produced 2 moles of bicarbonate), and assuming that no gypsum ($CaSO_4$) was dissolving (from the compost) or precipitating in the substrate (this

hypothesis is also valid for the sulfate reduction pathway as gypsum dissolution/precipitation would influence the SO_4^{2-} concentrations). Even though gypsum's saturation indexes (SI) obtained with the geochemical model PHREEQC were always negative (both in the pore-water and the effluent samples), it is important to consider that SI values in the open solution do not necessarily represent microenvironment values (where solute concentrations and pH can be substantially higher), and therefore the presence of gypsum cannot be entirely ruled out. Indeed, Rose et al. (2007) found small amounts of gypsum precipitating within Al precipitates and/or in patches on the limestone surface in four vertical flow ponds where PHREEQC modeling of the effluent indicated negative $CaSO_4$ SI values. In addition, some dissolved sulfide produced via SRB reduction could have been re-oxidized to sulfate within the reactors. Therefore the differences between influent and effluent SO_4^{2-} concentrations potentially slightly underestimated the amount of bicarbonate produced through the SRB reduction pathway.

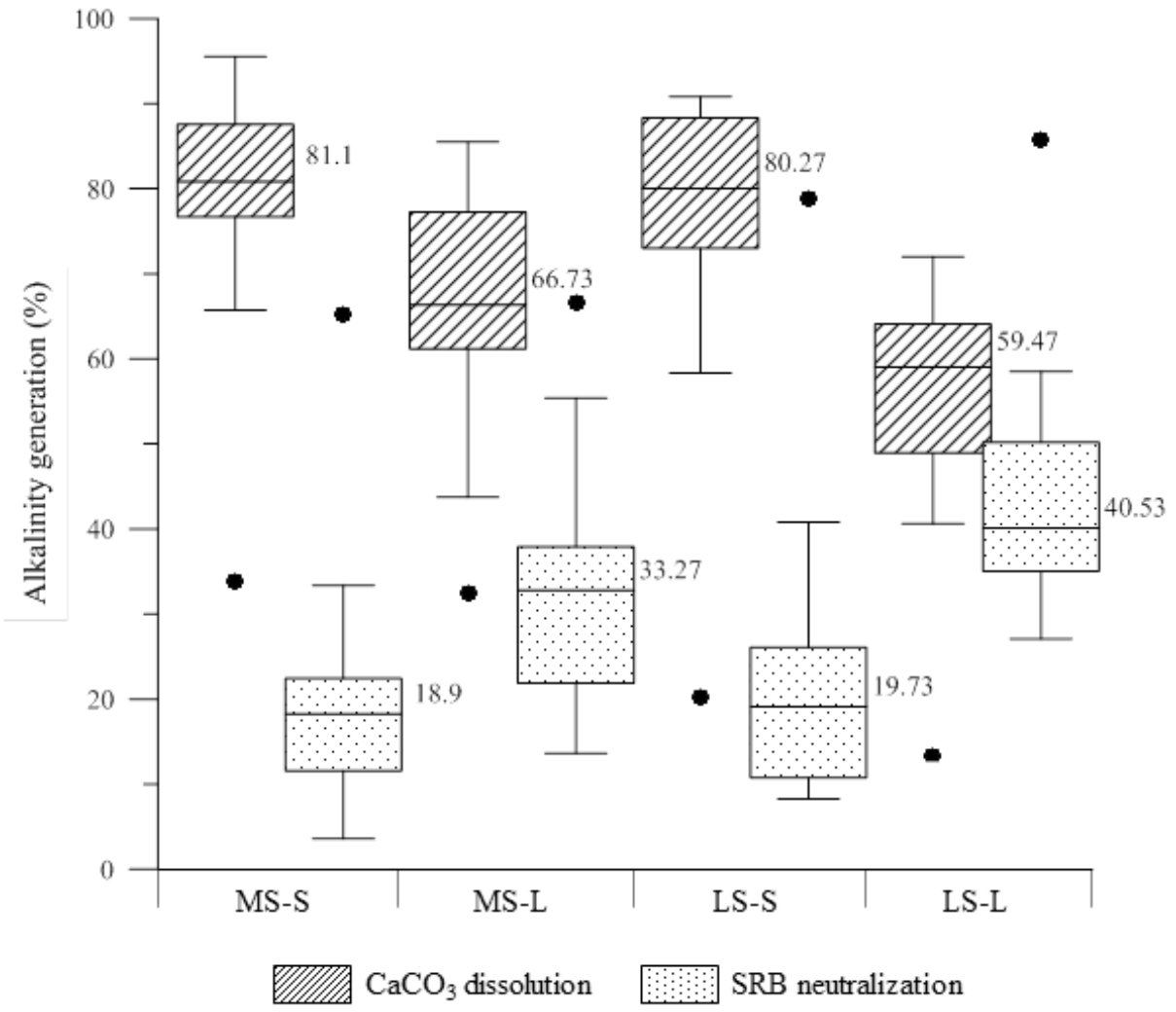


Figure 4-7. Alkalinity generation showing estimated contribution of $CaCO_3$ dissolution and SRB neutralization (box-plots show median, hinges at 25th and 75th percentiles, whiskers at 10th and 90th percentiles, and black dots are outliers defined as 1.5x IQR, numbers indicate median values).

Overall, the dissolution of $CaCO_3$ seemed to have generated a substantially greater fraction of alkalinity (59.47 to 81.10 %) compared to the SRB neutralization (18.90 to 40.53 %). These results are in contradiction to the total potential alkalinity generation presented in Chapter 3, which suggested that the alkalinity generation through the reduction of SO_4^{2-} should be the dominant process (estimations provided in Chapter 3 suggested that one might expect

approximately 70-80 % alkalinity generation via SRB neutralization and only 20-30 % through $CaCO_3$ dissolution). In reality, the assumptions made in Chapter 3 were oversimplifying the alkalinity generation processes and ultimately the rate of alkalinity production would be expected to be governed by numerous other parameters. These other controlling factors included the reaction kinetics (linked to the mineralogy of the alkaline materials), the influent MIW chemical signature (especially acidity, Fe, Al and SO_4^{2-} concentrations), a number of microbial and substrate properties (e.g. SRB concentrations, species diversity, biological activity, proportion of readily available substances, rate of cellulose and hemicellulose hydrolysis), and operational design parameters (e.g. HRT, flow design, temperature).

The two alkalinity generation pathways exhibited distinct behaviors. The calcium carbonate dissolution mechanism was mainly a function of the amount of acidity entering the systems with the $CaCO_3$ dissolution negatively influenced by HRT. The short HRT systems, which received more acidity compared to the long HRT systems, resulted in more alkalinity production via the $CaCO_3$ dissolution mechanism. This finding was consistent with well-known geochemical mechanisms relating a higher calcium carbonate solubility with a lower influent pH or a higher influent acidity (Morse and Arvidson 2002; Zipper and Skousen 2010). The alkalinity generated through $CaCO_3$ dissolution was also affected by the alkalinity source with the systems using mussel shell resulting in more alkalinity compared to the systems using limestone. This difference was especially noticeable for the long HRT systems. These observations were in agreement with the findings mentioned above (discussion on

Figure 4-6 and Table 4-3), and confirmed by the statistical analyses showing that the HRT had a small but significant influence on the $CaCO_3$ dissolution ($p < 0.05$), while the alkalinity source had a strong influence ($p < 0.001$). By contrast, the SRB neutralization mechanism was positively correlated to the HRT, with the longer HRT systems resulting in more alkalinity. This finding was in agreement with the sulfidogenesis process (i.e. the longer the HRT the more time the bacterial community had to degrade the substrate and reduce the sulfates). The SRB neutralization might also have been affected by the alkalinity source as reactors containing mussel shell yielded slightly less alkalinity compared to the reactors using limestone (Figure 4-7). These observations were confirmed by the two-way ANOVA analyses showing that the HRT had a very significant influence ($p < 0.001$) on the alkalinity generation via the SRB neutralization pathway, while the alkalinity source had none ($p = 0.816$).

4.3.2.4 Sulfate removal and sulfide generation

Figure 4-8 shows the influent and effluent sulfate and sulfide concentrations and Figure 4-9 shows the sulfate removals both as a relative percentage (%) and as a rate of mole per m^3 of substrate per day ($mol/m^3/d$). Table 4-4 also shows the sulfate removal values both in relative percentage (%) and in mole per m^3 of substrate per day ($mol/m^3/d$).

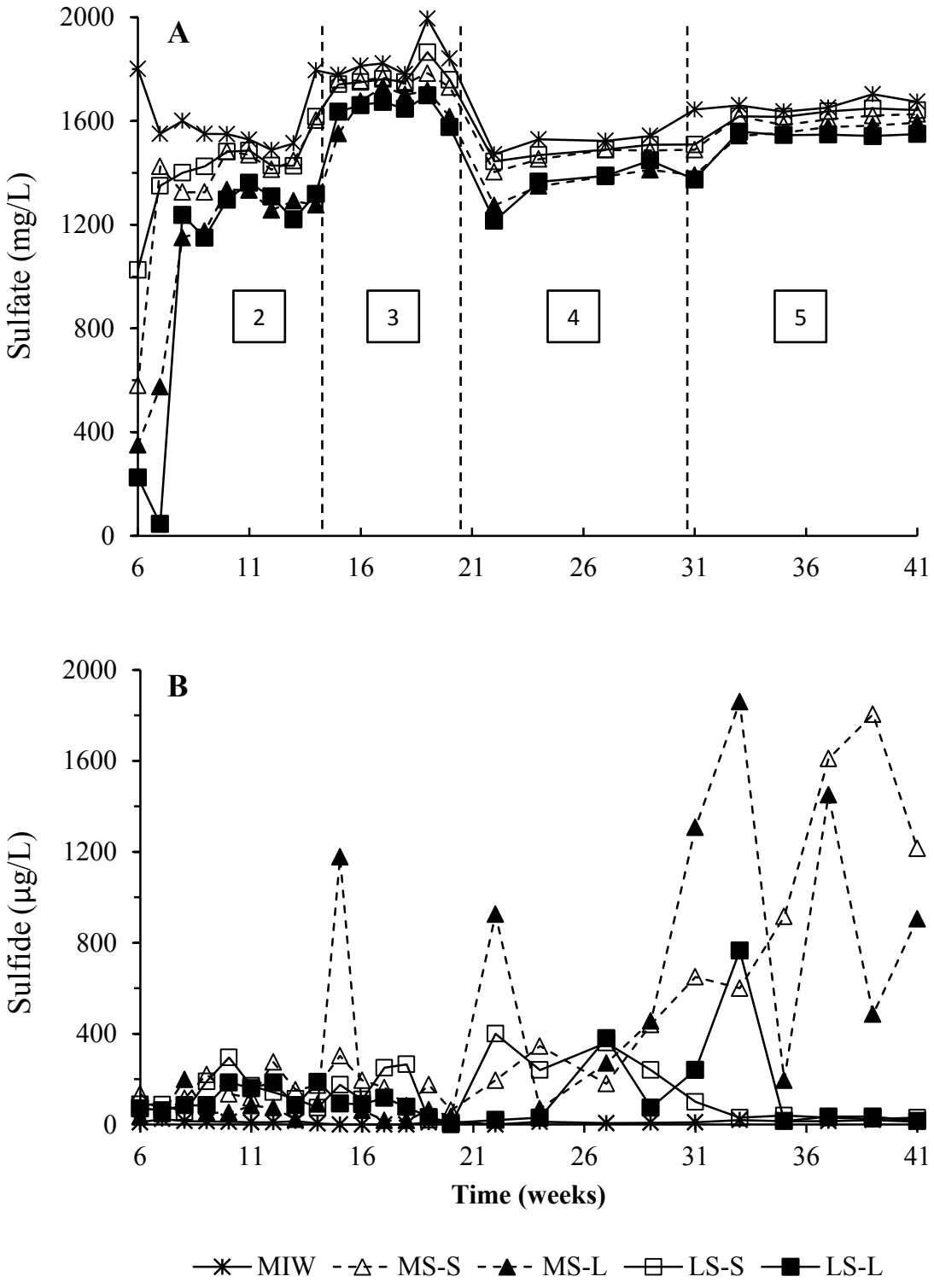


Figure 4-8. (A) Sulfate (mg/L), and (B) sulfide (µg/L) concentrations over the 41 weeks of treatment. Vertical dash lines indicate when a new batch of MIW was used.

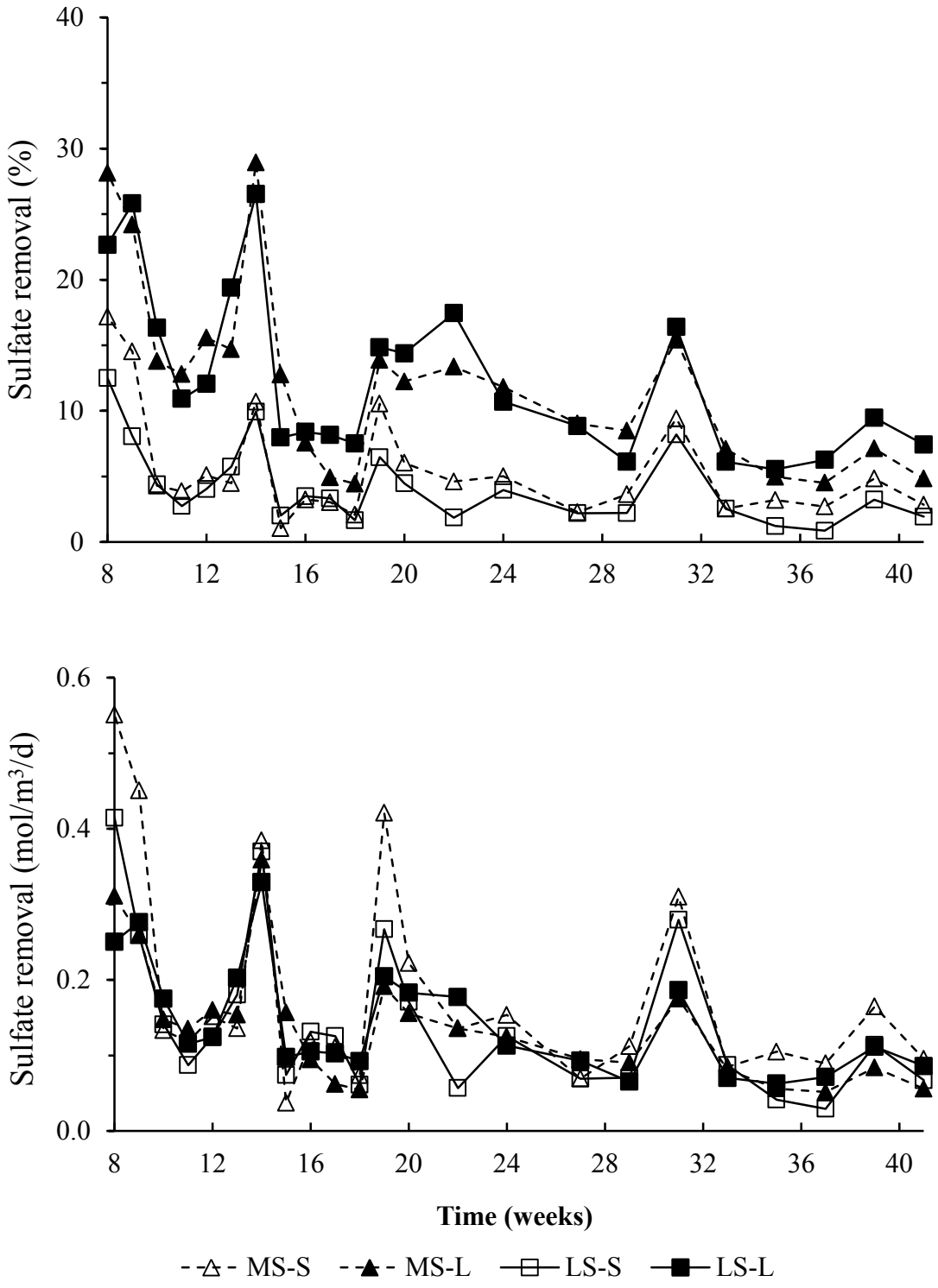


Figure 4-9. (A) Sulfate removal in percentage (%), and (B) sulfate removal in mole per m^3 of substrate per day (mol/m^3/d) over the 41 weeks of treatment.

Table 4-4. Sulfate removal in % and in mol/m^3/d (median and range values).

Sulfate removal (%)	MS-S	MS-L	LS-S	LS-L
Week 8 - 20	4.5 (1.0-17.2)	13.8 (4.4-28.9)	4.4 (1.7-12.5)	14.4 (7.5-26.5)
Week 22 - 41	3.4 (2.3-9.4)	7.8 (4.5-15.4)	2.2 (0.9-8.2)	8.1 (5.5-17.4)
Week 8 - 41	4.3 (1.0-17.2)	12.2 (4.4-28.9)	3.3 (0.9-12.5)	10.7 (5.5-26.5)
Sulfate removal (mol/m^3/d)				
Week 8 - 20	0.14 (0.04-0.55)	0.16 (0.05-0.36)	0.14 (0.06-0.41)	0.18 (0.09-0.33)
Week 22 - 41	0.10 (0.07-0.31)	0.09 (0.05-0.17)	0.07 (0.03-0.28)	0.09 (0.06-0.19)
Week 8 - 41	0.13 (0.04-0.55)	0.14 (0.05-0.36)	0.13 (0.03-0.42)	0.11 (0.06-0.33)

Similar to the alkalinity generation, sulfate removal largely fluctuated until it reached a steady-state behavior approximately at week 8. Sulfate concentrations and removal rates also fluctuated following changes in sulfate concentration in the influent MIW (sulfate concentration changed across the different batches of MIW used; Figure 4-8 A). While the long HRT systems continuously removed more sulfate compared to the short HRT systems, no constant trend was noticeable between the mussel shell and the limestone containing SRBR (Figure 4-8 A, Table 4-4). Again this observation was in accordance with the sulfidogenesis principle (i.e. the longer the MIW stays in contact with the substrate and its associated micro fauna, the longer the bacteria had to reduce the sulfate into sulfide). These results were confirmed by the statistical analyses showing that the differences of effluent sulfate concentrations between HRT were significant ($p < 0.01$), while the differences of effluent sulfate concentrations between alkalinity source were not ($p = 0.986$).

Figure 4-8 (B) shows dissolved sulfide concentrations in the effluent samples. The sulfide concentrations in the effluent samples were generally very low (usually < 1 mg/L) and constantly below 25 μg/L in the influent MIW. Nevertheless, a strong sulfide odor was detected during all sampling events indicating that a substantial amount of H_2S gas escaped the reactors as mentioned earlier (see experimental design section in Chapter 3). This observation was in accordance with the fact that at the observed pH, temperature, and pressure ranges, most of the hydrogen sulfide was present in the water as insoluble H_2S gas (Carroll and Mather 1989). Overall, little differences were observed between the four systems and while no clear trend could be visually noticed (Figure 4-8 B), the ANOVA analyses showed that the alkalinity source had a strong influence on the effluent sulfide concentration ($p < 0.001$), but not the HRT ($p = 0.842$). Despite the statistical analyses, these results should be taken with care because some sulfide was likely lost as a result of air oxidation (e.g. the samples were not filtered to prevent filtering out colloidal sulfides, and were instead allowed to sit covered for approximately 5 minutes to let the suspended solids settle down to the bottom of sample cells before being analyzed with the HACH spectrophotometer).

When comparing the sulfate removal on a daily molar volumetric removal basis (Figure 4-9 B, Table 4-4), the differences between short and long HRT as well as between mussel shell or limestone containing SRBR were less noticeable and not statistically significant ($p = 0.274$ for the HRT, and $p = 0.432$ for the alkalinity source). Several studies reported sulfate removal ranging between approximately 0.11 to 0.63 mol/m^3/day, and between 12 to 58 % (Béchard et al. 1994; Chang et al. 2000; Dvorak et al. 1992; Figueroa et al. 2007; Gibert et al. 2004; Thomas and Romanek 2002a; Waybrant et al. 2002). In addition, based on a large number of

studies, Gusek (2002) recommended using a value of 0.3 mol/m^3/day when designing a new SRBR. Despite the fact that pore-water pH and ORP were both within the desirable range for SRB to metabolize (pH > 5.5 and ORP < -100 mV; (Postgate 1979), the sulfate removal rates in the present study mostly stayed below the 0.3 mol/m^3/d design value. Several hypotheses can explain these lower results: (1) a poor initial SRB establishment, due to the substrate's low nitrogen content, could indicate that the microbial community stayed small in terms of microbial mass; (2) despite the 3-week acclimation period, the neutrophilic SRB naturally inoculated from the compost and adapted to a neutral pH might have suffered from the influent low pH (Whitehead et al. 2005). This second hypothesis was supported by the fact that following the introduction of MIW batch #3 at week 14 (MIW batch #3 had the highest acidity and the highest metal concentrations), the sulfate reduction (both in terms of % and mol/m^3/d) drastically decreased. The use of acidophilic SRB (e.g. using MIW impacted creek sediments or spent substrate from a mature SRBR as an inoculum) might have helped in maintaining an active SRB population and sustained higher sulfate removal rates. Other studies showed that a rapid increase in the influent MIW acidity and/or metal concentrations can have strongly negative and sometime irreversible repercussions on the sulfate reduction rates (Drury 1999). Finally, the 2-month interruption period did not resulted in an increase of the sulfate reduction rates as suggested by Eger and Wagner (2002).

4.3.2.5 Metal removal

Treatment performances were first assessed using the percentage of dissolved metals removed, as defined by Wieder (1989), (Equation 4.2, Table 4-5).

$$\text{Treatment efficiency (\%)} = (C_{in} - C_{out})/C_{in} \times 100 \quad (4.2)$$

Where:

C_{in} and C_{out} = inflow and outflow metal concentrations (mg/L).

Removal efficiencies were also assessed using the area-adjusted removal rate as defined by Hedin et al. (1994) (Equation 4.3, Table 4-8). Because in SRBR design the depth is often a more important dimension than the length, width, or radius (in case of a column-shaped reactor), reporting results in term of volume-adjusted removal rate ($g/m^3/day$) by substituting volume (m^3) to area (m^2) in Equation 4.3 seemed more appropriate. Both area- and volume-adjusted removal rates are shown in Table 4-8.

$$R_A = Q\,(C_{in} - C_{out})/\,A \quad (4.3)$$

Where:

R_A = area-adjusted removal rate ($g/m^2/day$),

C_{in} and C_{out} = inflow and outflow metal concentrations (mg/L),

Q = flow rate (m^3/day),

A = area (m^2).

Table 4-5. Treatment efficiencies in terms of dissolved metal removal (median and range values in %).

	Al	Fe	Mn	Cu	Zn	Ni	Cd
Mussel shell short HRT							
Week 6-20	99.49	91.96	29.65	89.64	99.45	83.01	99.01
Week 22-41	98.62	87.46	7.06	70.00	99.43	67.75	97.87
Week 6-41	99.21 (85.4-99.7)	90.87 (79.2-95.9)	15.89 (-1.6-68.0)	84.34 (13.9-97.7)	99.45 (63.7-99.8)	82.12 (55.6-96.9)	98.63 (86-100)
Mussel shell long HRT							
Week 6-20	99.73	95.18	55.17	91.76	99.64	95.25	99.38
Week 22-41	99.66	96.70	30.62	80.01	99.68	96.44	99.99
Week 6-41	99.71 (98.6-99.9)	95.24 (91.8-99.3)	38.76 (16.4-80.3)	86.40 (52.7-98.2)	99.65 (95.2-99.9)	95.91 (70.7-99.3)	99.83 (93.1-99.9)
Limestone short HRT							
Week 6-20	99.00	86.51	19.84	87.39	99.09	81.71	98.09
Week 22-41	80.31	80.85	6.54	16.24	65.05	51.87	80.57
Week 6-41	97.57 (32.9-99.6)	83.94 (68.7-99.6)	9.15 (-12.7-66.1)	77.78 (-17.1-95.6)	92.27 (34.9-99.7)	68.19 (19.8-93.7)	97.39 50.7-99.8)
Limestone long HRT							
Week 6-20	99.37	91.78	47.13	91.22	96.12	82.74	98.75
Week 22-41	97.48	86.22	17.77	59.17	95.32	69.18	97.73
Week 6-41	99.08 (91.7-99.8)	90.62 (69.1-98.9)	26.60 10.6-77.6)	84.61 (3.9-96.8)	96.12 (78.5-99.7)	81.15 (51.9-93.4)	98.10 (71.9-99.9)

Over the 41 weeks of treatment, each system had high metal removal efficiencies for each metal (except Mn) with median removal values ranging between 77.78 and 99.83 %. The overall order of metal removal efficiency across all four systems was Al > Cd > Zn > Fe > Cu > Ni >> Mn, and the order of system's efficiencies was MS-L > MS-S > LS-L > LS-S (Table 4-5). Generally, systems operating at a longer HRT removed a higher percentage of metals than systems operating at a short HRT. Similarly, reactors containing mussel shell removed a higher percentage of metals than those containing limestone. MS-L was constantly the most effective reactor, removing on average 96.1 % of all metals (except Mn), while LS-S was the less efficient system removing only 86.2 % of metal (except Mn). In addition, system MS-S removed on average more metal (92.4 %, except Mn) than system LS-L (91.6 %, except Mn), suggesting that the alkalinity source had a greater influence on the treatment performance compared to the HRT.

The 2-way ANOVA statistical results examining the metal removal (%) as a function of both the HRT and the alkalinity source are summarized in Table 4-6. These statistical tests indicated that the HRT had a significant influence on the removal of each metal, including Mn, for which the significance was found to be very strong ($p < 0.001$). Similarly, the alkalinity source was found to have a significant influence on the removal of each metal. The higher removal efficiencies reported for systems using mussel shell and/or operating on a longer HRT concurred with the lower ORP, the higher pH, and the higher alkalinity data reported for these systems.

Table 4-6. P-values of 2-way ANOVA for dissolved metal removal (in %) as a function of the HRT and the alkalinity source.

Metal removal	HRT	Alkalinity source
Fe	***	***
Al	**	**
Mn	***	*
Zn	**	***
Ni	***	***
Cu	*	*
Cd	*	**

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Although a direct comparison between the reactors' effluent metal concentrations and the trigger values (TVs) presented in Table 4-1 is not truly meaningful (i.e. TVs can only be compared with in-stream concentrations where dilution and mixing is occurring), a relative evaluation of the SRBR's effluent metal concentrations is provided in Table 4-7. Again, it can be seen that system MS-L was the best performing system containing only Fe, Cu, Ni and Mn in concentrations exceeding the Guidelines for Freshwater Aquatic Ecosystems protection and only Fe and Mn exceeding the Guidelines for Recreational Purposes. Taking into account that these effluent concentrations would likely be greatly diminished once reasonably diluted with surface waters, it can be relatively safely assumed that only Mn would still be present in concentrations greater than the TVs (0.1 and 3.6 mg Mn/L for the Guidelines for Freshwater Aquatic Ecosystems and the Guidelines for Recreational Purposes, respectively).

Table 4-7. Median effluent dissolved metal concentrations (mg/L) and comparison with trigger values (a red shading indicates that metal concentrations were above both the Guidelines for Freshwater Aquatic Ecosystems and the Guidelines for Recreational Purposes, a green shading indicates that metal concentrations were below both Guidelines, and a grey shading indicates that metal concentrations were above the Guidelines for Freshwater Aquatic Ecosystems, but below the Guidelines for Recreational Purposes).

	MS-S	MS-L	LS-S	LS-L
Fe	2.4	0.7	2.7	1.7
Al	0.1	0.04	0.4	0.1
Mn	13.5	9.9	14.8	11.8
Zn	0.02	0.01	0.4	0.2
Cu	0.03	0.02	0.04	0.03
Ni	0.2	0.04	0.4	0.2
Cd	0.00005	0.00003	0.0001	0.00005

Figure 4-10 and Figure 4-11 show dissolved metal removal (%) over time, and Figure 4-12, Figure 4-13, and Figure 4-14 show the total amount of metals (mg/day) retained in the SRBR's substrates and discharged with the effluents.

From these figures, two general observations can be made: (1) metal removal trends differed between metals and between systems; and (2) total metal removal fluctuated but generally decreased over time indicating that the metal removal processes also changed over time. Overall, during week 6 to 20, metal removal was effective in all four treatment systems for all metals of concern (except Mn). During the second 5-month treatment period (week 22 to 41), metal removal was less effective in all SRBR systems, and especially in system LS-S for which the metal removals became erratic. Irregular removal trends, as observed for system

LS-S, generally indicate that a system is performing under stress and/or malfunctioning (Gusek and Wildeman 2002b). Individual metal removal behaviors are discussed below, and a geochemical modeling investigation is presented and discussed at the end of Chapter 5.

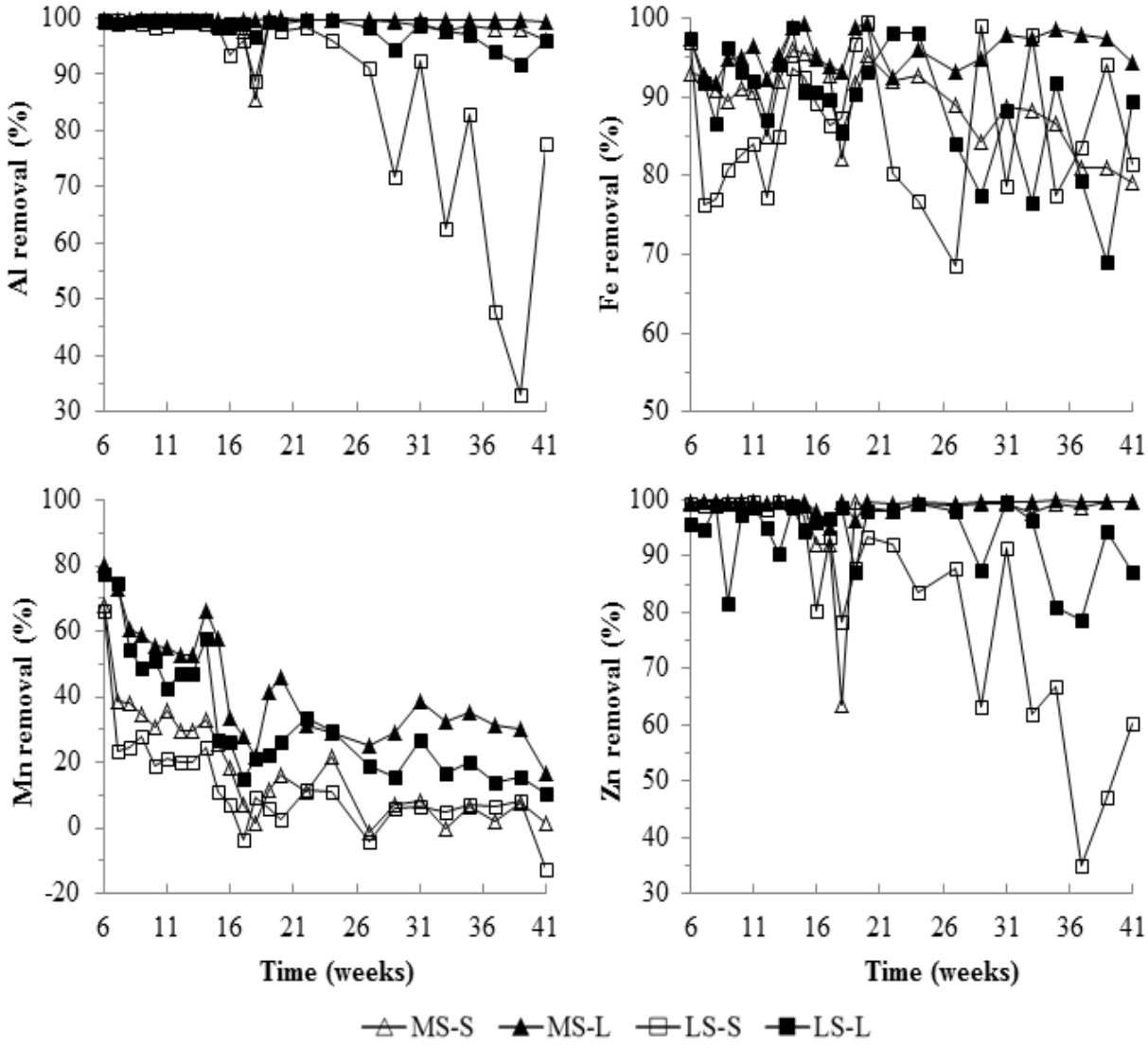


Figure 4-10. Al, Fe, Mn and Zn removal (%) over time (note the different vertical scales).

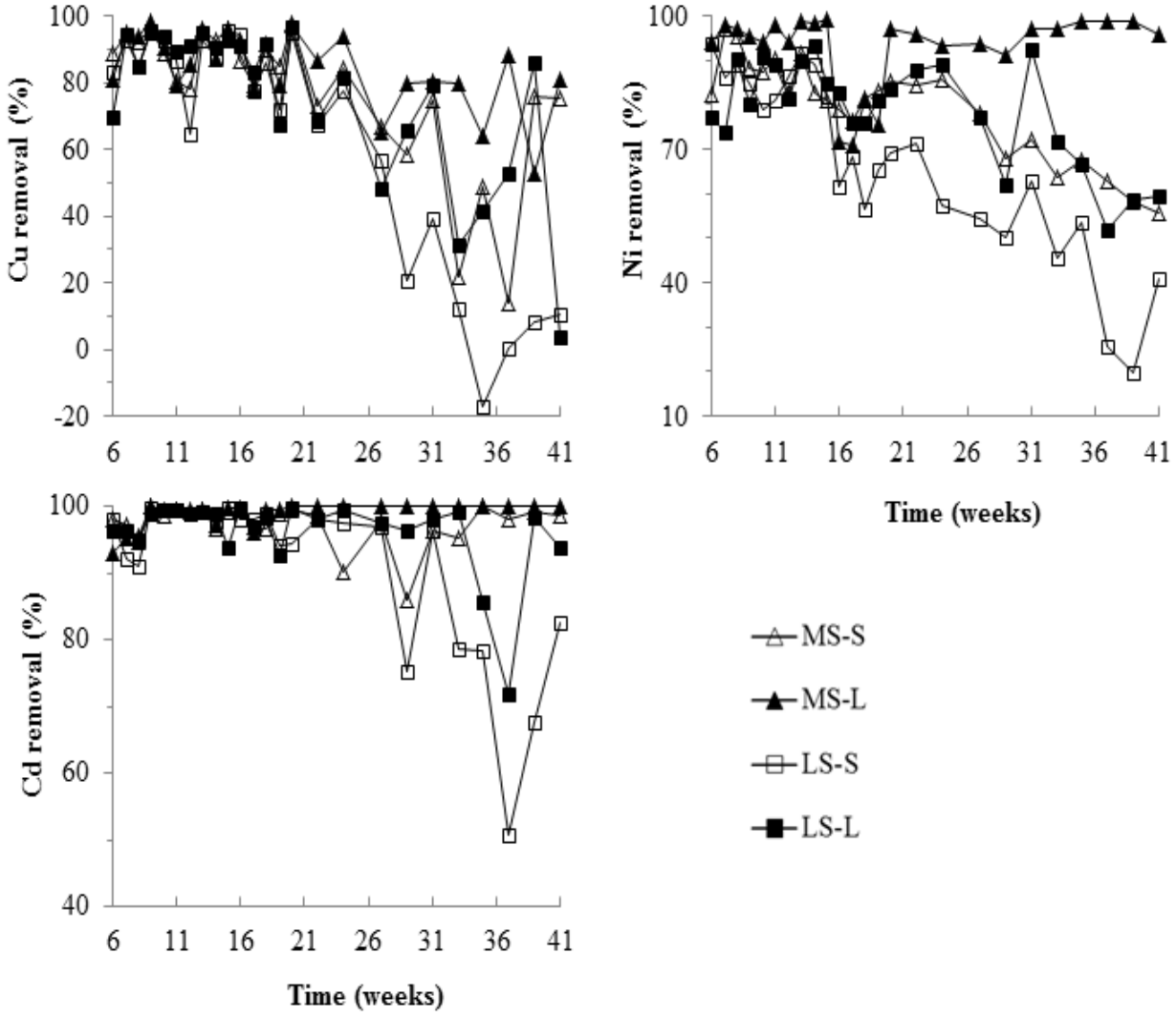


Figure 4-11. Cu, Ni and Cd removal (%) over time (note the different vertical scales).

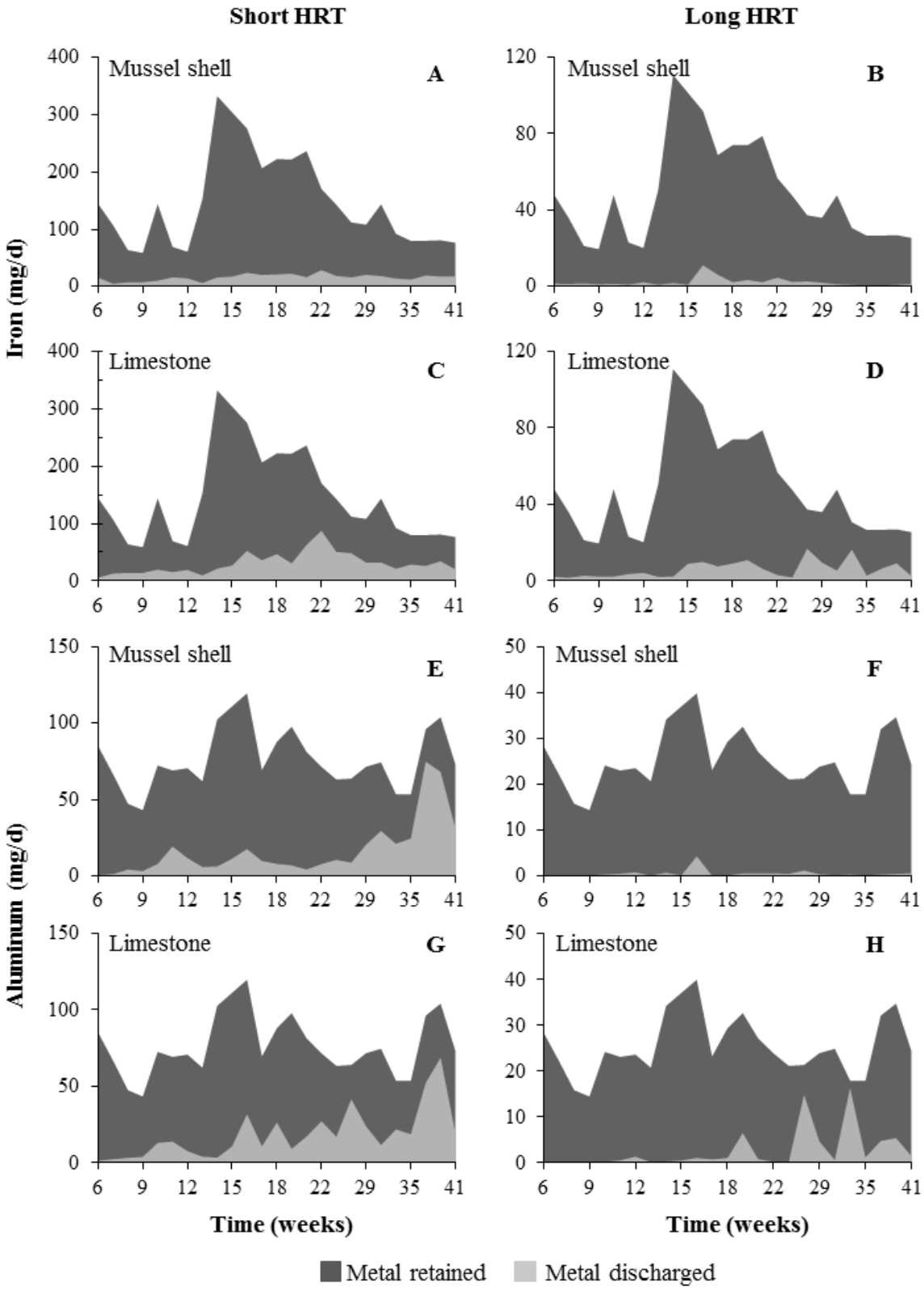


Figure 4-12. Total Fe and Al retention in mg/d (note the different vertical scales).

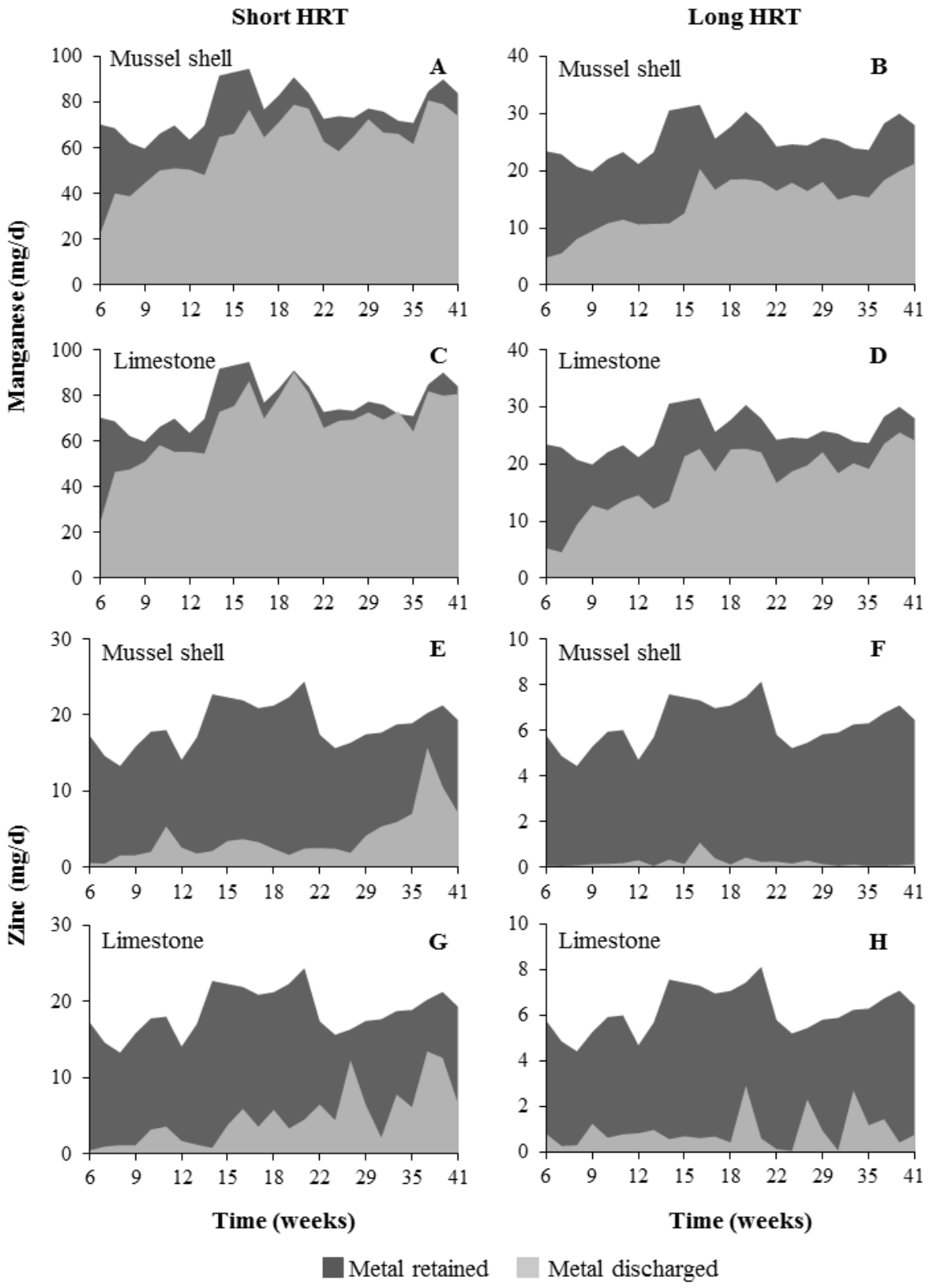


Figure 4-13. Total Mn and Zn retention in mg/d (note the different vertical scales).

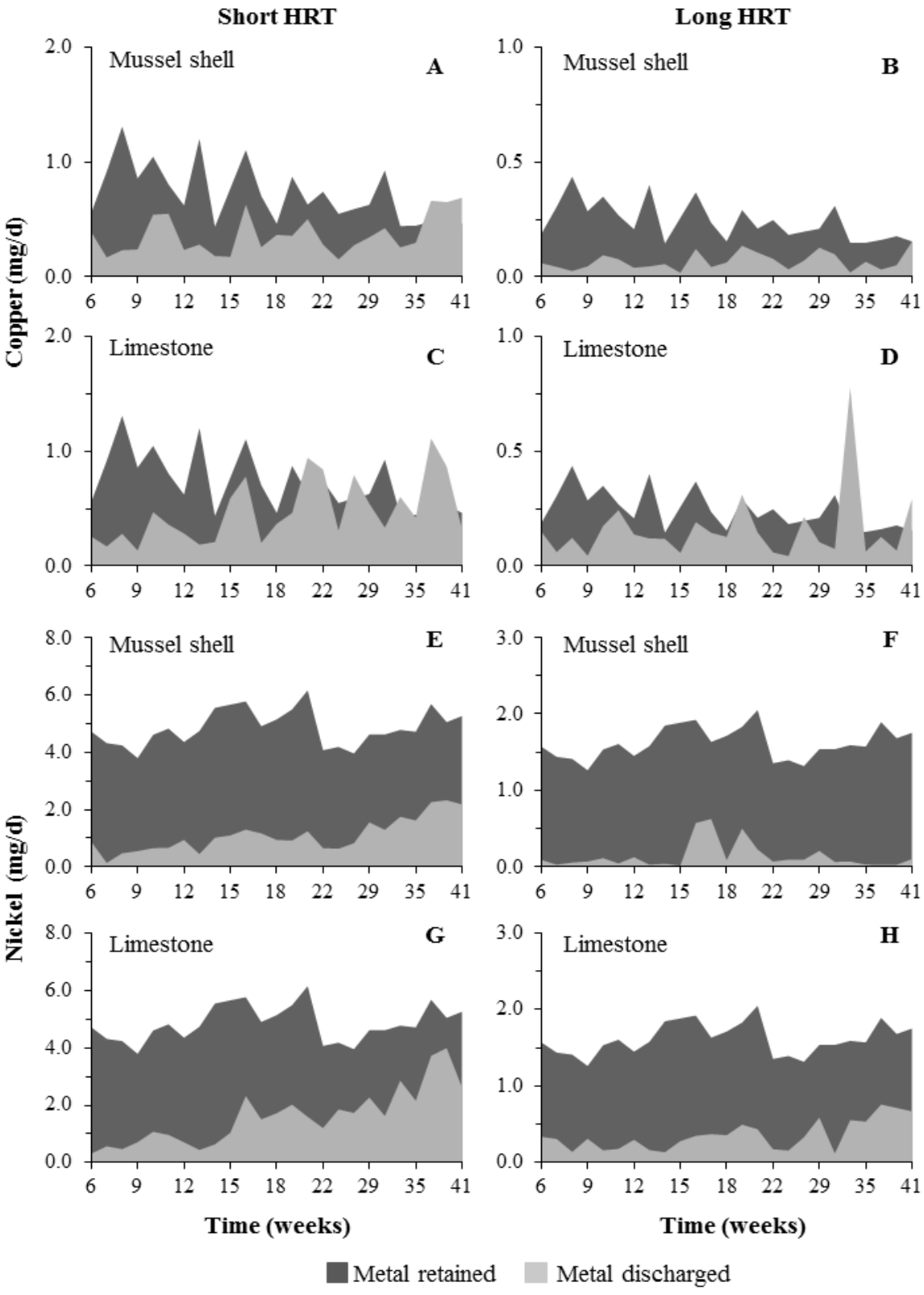


Figure 4-14. Total Cu and Ni retention in mg/d note the different vertical scales).

Iron:

In reducing systems, Fe is suspected to be removed mainly as iron sulfides, but depending on the ORP, pH and alkalinity, iron hydroxides and iron carbonates may precipitate as well (ITRC 2012; Younger et al. 2002). In the present study, Fe removal ranged between 84 to 95 %. These results were comparable with previously reported Fe removal efficiencies. For example, authors reported Fe removal in SRBR and other similar systems (e.g. vertical flow wetlands) ranging from 14.3 to 96.8 % (Rose and Dietz 2002), 51.5 to 99.3 % (Choudhary and Sheoran 2011), 82 to 83 % (Jong and Parry 2003), 94 to 99 % (McCauley et al. 2009). Fe removal seemed to be independent of the loading (at the concentrations used in this study). For example, while Fe loading increased three-fold at week 13 (changing from MIW batch #2 to batch #3), the total Fe discharge only increased by a few mg/d (Figure 4-12). Although systems containing mussel shell and/or operating at a longer HRT significantly retained more Fe than systems containing limestone and/or operating at a shorter HRT (Table 4-5, Table 4-6), the four treatment designs did not show great differences in the total amount of Fe discharged (Figure 4-12). This general behavior supported the hypothesis that the main Fe removal mechanism was precipitation as iron sulfides with the possible precipitation of iron (oxy)hydroxides (e.g. hematite, goethite, ferrihydrite), especially in the short HRT systems. During the second 5-month treatment period, Fe removal started exhibiting signs of decreased efficiency for system LS-S only. The fact that during the second 5-month treatment period, the sulfate reduction rates, the pH, and the alkalinity all concurrently decreased, and the ORP increased, supported the hypothesis that Fe was removed as sulfide and as (oxy)hydroxides.

Aluminum:

Al removal in SRBR is a rather straight forward mechanism as Al is assumed to be retained as an amorphous hydroxide (e.g. $Al(OH)_3$) or in the crystalline form of gibbsite once the pH is raised above 4.5 (Hedin et al. 1994). Additionally, depending on the pH and the sulfate concentration, precipitation of hydroxysulfates such as basaluminite or ettringite (e.g. $Al_4(OH)10(SO_4)\cdot 4H_2O$, $Ca_6Al_2(OH)_{12}(SO_4)_3\cdot 26H_2O$) is another possible removal mechanism (Stumm and Morgan 1996; Watzlaf et al. 2004). In the present study, Al removal ranged between 97.6 and 99.7 % (Table 4-5), and the concentration of Al in the effluent was constantly below 1 mg/L for all systems apart from reactor LS-S. Again these results were in accordance with published data where Al removal ranged from 25 to 100 % (Rose and Dietz 2002), 100 % (Dvorak et al. 1992), 95.9 to 100 % (McCauley et al. 2009). In reactor LS-S, Al concentrations in the effluent ranged between 1.1 and 13.5 mg/L during week 27 to 41 (effluent pH during the same period ranged from 3.8 to 5.7), confirming that Al removal was strongly influenced by the pH. Similarly to Fe, Al removal was significantly influenced by both the HRT and the alkalinity source (i.e. SRBR containing mussel shell and/or operating at a longer HRT resulted in higher Al removal, Table 4-6).

Manganese:

In the present study, Mn removal ranged between 9.2 and 38.8 % (median values weeks 6 to 41). During weeks 6 to 20, Mn removal in system MS-L even reached 55.2 %. These removal efficiencies reported for reactor MS-L were relatively high compared to typical values found in the literature (e.g. poor Mn removal and even Mn export is often reported in SRBR studies). By contrast with Fe and Al, Mn showed a constant decline in removal efficiency over time,

and export of Mn was observed in the short HRT reactors (Figure 4-10). In addition, important differences were observed between each system.

The removal efficiencies (Table 4-5, Figure 4-10 and Figure 4-13) showed that reactors containing mussel shell and/or operating at a longer HRT retained more Mn, and these observations were supported by the statistical analyses showing a significant influence of the HRT ($p < 0.001$) and of the alkalinity source ($p < 0.05$, Table 4-6). Assuming that under the reducing conditions observed in this study, Mn removal as oxide, hydroxide, or sulfide was unlikely or strictly limited to microenvironments within the substrate, adsorption onto the organic matter and precipitation as a carbonate (e.g. rhodochrosite) were the most likely removal mechanisms (Robinson-Lora and Brennan 2010a; Waybrant et al. 1998). These hypotheses were supported by: (1) the decrease in Mn removal over time suggesting that adsorption might be an important retention mechanism (i.e. as adsorption sites reached capacity, less Mn was being removed; and/or Mn previously adsorbed could have been displaced by the action of other cations such as Zn, Cd and Cu which can bond more strongly to humic substances (Kerndorff and Schnitzer 1980); (2) the work of Cubillas et al. (2005b), which showed that aragonite affords more adsorptive surface area than calcite, (3) the higher Mn removal correlated with the higher alkalinity in long HRT systems (correlation between Mn removal and alkalinity showed $R^2 = 0.76$ for MS-L and $R^2 = 0.88$ in LS-L), (4) PHREEQC modeling showing that rhodochrosite was slightly oversaturated in all systems' effluents during the first 5-month period, but only oversaturated in system MS-L during the second 5-month treatment period (for week 6 to 20, SI values for $MnCO_3$ were: 0.4 (MS-S), 0.85 (MS-L), 0.03 (LS-S), and 0.47 (LS-L); for week 20 to 41, SI values were: -0.37 (MS-S),

0.12 (MS-L), -2.05 (LS-S), and -0.85 (LS-L)); and (5) the work of Lebron and Suarez (1999) which showed that supersaturated solution of $MnCO_3$ could be metastable for 3 days or more, indicating that the HRT had a crucial influence on the precipitation of rhodochrosite. Finally, a few studies (Robinson-Lora and Brennan 2010b) indicated that Mn-phosphate minerals (e.g. $MnHPO_4$) could precipitate within a SRBR. This last hypothesis was supported by the PO_4^{3-} results presented above and is discussed later in Chapter 5 along with the geochemical modeling results.

Copper, Zinc, Nickel and Cadmium:

In reducing and anaerobic systems, other divalent metals like Cu, Zn, Ni, and Cd are expected to be principally removed via sulfide precipitation (Younger et al. 2002) and possibly co-precipitation with Fe- and Al-(oxy)hydroxides (Gibert et al. 2005). Precipitation of insoluble phosphate minerals is also a potential sink for these metals (ITRC 2012), while carbonate and hydroxide precipitations are only likely to happen in microenvironments where the pH is at least above 7 and the metal concentrations are higher.

Zn and Cd had high and similar removal efficiencies ranging from 92.3 to 99.7 % (Zn) and 97.4 to 99.8 % (Cd), while Cu and Ni both display lower removal efficiencies (77.8 to 86.4 % for Cu and 68.2 to 95.9 % for Ni). The values for Zn, Cd and Ni were roughly within typical values reported in the literature (35.1 to 99.8 % Zn, 17.9 to 99.1 % Ni), while the values for Cu were below (85.7 to 100 % Cu) (Das et al. 2012; Jong and Parry 2003; Choudhary and Sheoran 2011). In addition, the removal of Zn and Cd was relatively constant over the 41

weeks of treatment in all reactors apart from LS-S, but the removal of Cu and Ni declined in all reactors during weeks 20 to 41 (Figure 4-11). After a sharp decline in Ni removal at week 14 in reactor MS-L, likely explained by the lower influent pH and the higher influent metal concentrations (following the introduction of MIW batch #3), Ni removal restored to previous higher values.

Several authors found that the removal of Cu, Zn, Ni, and Fe followed the solubility product (K_s) values of the respective metal sulfides (Sahinkaya and Gungor 2010; Jong and Parry 2003). Although the K_s values used in these studies slightly differ, the same removal order was respected: Cu > Zn > Ni >> Fe. This observation was not confirmed in the present study as more Zn and Fe were retained than Cu or Ni. Again this finding suggested that sulfide precipitation was not the only metal removal mechanisms taking place in the SRBR.

Table 4-8. Area- and volume-adjusted dissolved metal removal rates.

	Mussel shell short HRT		Mussel shell long HRT		Limestone short HRT		Limestone long HRT		Published removal rates	
	Area-adjusted ($g/m^2/d$)	Volume-adjusted ($g/m^3/d$)	Area-adjusted ($g/m^2/d$)	Volume-adjusted ($g/m^3/d$)	Area-adjusted ($g/m^2/d$)	Volume-adjusted ($g/m^3/d$)	Area-adjusted ($g/m^2/d$)	Volume-adjusted ($g/m^3/d$)	Area-adjusted ($g/m^2/d$)	Volume-adjusted ($g/m^3/d$)
Al	0.90	2.92	0.30	0.97	0.85	2.62	0.30	0.96	1.7-3.2[a]	-
Fe	1.09	3.52	0.41	1.32	1.17	3.8	0.33	1.08	0.8[b]	-
Mn	0.16	0.52	0.12	0.40	0.10	0.32	0.094	0.30	0.01[b]	-
Cu	0.007	0.024	0.002	0.008	0.007	0.022	0.002	0.008	10[c]	-
Zn	0.253	0.817	0.086	0.278	0.216	0.695	0.078	0.250	0.11[d]	0.79[f]
Ni	0.046	0.147	0.018	0.059	0.043	0.139	0.016	0.051	2[e]	-
Cd	1.66E-04	5.36E-04	5.51E-05	1.77E-04	1.57E-04	5.04E-04	5.42E-05	1.75E-04	0.02[e]	-

[a] = Hedin et al. (1994)
[b] = Heal and Salt (1999)
[c] = PIRAMID Consortium (2003)
[d] = Kadlec and Wallace (2009)
[e] = Ettner (1999)
[f] = Gandy and Jarvis (2012)

The following order of metal removal efficiency was generally observed for both area- and volume-adjusted removal rates: Fe > Al > Mn > Zn > Ni > Cu > Cd (Table 4-8). Overall, the short HRT systems always outperformed long HRT systems, indicating that metal loading was probably a limiting factor, and that the long HRT systems possibly performed below capacity. While Fe, Mn and Zn removal rates were slightly above typical rates reported in the literature, Cu, Ni, and Cd were well below. The low influent pH could explain these sub-optimal removal rates as most of the literature values reported were derived from field and laboratory experiments using MIW with pH > 3. Moreover, depending on reactor design, substrate materials, microbial development as well as influent pH and metals loadings, large disparities in treatment performances have been reported (Mayes et al. 2009), thus limiting comparison of published removal rates using different operational designs.

Finally, a comparison (in moles) between the total amount of sulfate reduced and the total amount of metals retained (except Al and Mn, which were not assumed to form sulfides under the experimental conditions) from week 8 to 41 (steady-state conditions) showed that the number of moles of sulfate removed exceeded the number of moles of metals removed in each of the four systems, thus indicating that each system was likely to have generated enough sulfides to remove all the metals as sulfides (excluding Al and Mn) and supported the hypothesis that metal loading might be a limiting factor to the metal sulfide removal pathway, again possibly explaining the small removal rates reported in Table 4-8.

4.4 Conclusions

In general, each of the four SRBR systems successfully treated MIW over the first 5 months of treatment as indicated by most of the monitored parameters. During the second 5-month period (after two months of interruption), metal and sulfate removals generally slightly declined in all four systems, and alkalinity generation dropped in all systems apart from MS-L. This indicated that, in the present study, the 2-month interruption period did not result in a noticeable improvement of the treatment performances (e.g. increase in sulfate reduction rates, effluent pH, or in metal removal) as suggested by other studies (Eger and Wagner 2002; Whitehead et al. 2005). Sulfate and/or the EAS depletion may have occurred during the resting period, resulting in a decline of the microbial population mass, thus in a diminution of the treatment performances. Overall only the sulfate reduction was reported below the typical usual values of 0.3 mol/m^3/d found in the literature (Gusek and Wildeman 2002a; ITRC 2012; Wildeman et al. 2006). Relatively high Mn removal (median value for week 6 to 41 was 38 %) occurred in system MS-L and was attributed to both Mn-carbonate precipitation and adsorption onto the organic matter.

Data analysis showed that the dissolution of calcium carbonate was responsible for 60 to 80 % of the total alkalinity generation, the rest being attributed to the reduction of sulfate and the concomitant generation of bicarbonate. This finding contradicted the hypothesis made in Chapter 3 (based on the complete use of C and Ca in the substrate materials) suggesting that the sulfate removal pathway should be responsible for most of the alkalinity generation. Ultimately, the reaction kinetics and a number of substrate and design properties likely controlled the alkalinity

generation. Furthermore, the mussel shell mixtures yield substantially more alkalinity compared to the limestone, at both HRT.

Generally, reactors operating at a longer HRT_n and/or containing mussel shells resulted in better metal removal and alkalinity generation compared to the systems operating at a shorter HRT_n and/or containing limestone. Statistical analyses summarized in Appendix A-2 showed that the HRT had more influence on the reactors' performances compared to the alkalinity source, with the reactors operating at a longer HRT_n generally resulting in a significantly better treatment efficacy for the following parameters: pH, ORP, D.O., alkalinity, sulfate reduction, organic carbon generation and metal removal. Nevertheless, the alkalinity source also had a positive influence on the treatment efficiency with the reactors using mussel shell instead of limestone resulting in significantly better performances in terms of pH, alkalinity generation, and metal removal.

Possible reasons for the better treatment observed in the reactors containing the mussel shell are thought to be related to its unique mineralogical composition made of both aragonite and calcite. The material and grain size, shape and greater reactive surface area of the mussel shell might also have beneficiated the alkalinity generation and the removal of metals through both carbonate precipitation in higher pH microenvironments close to the shell fragments and adsorption onto chitin-related materials associated with the shells. In addition, organic matter associated with the mussel shell and nitrogen contained within the shell matrix might have supported the microorganism consortium responsible for the substrate biodegradation and the sulfidogenesis. However, this last hypothesis seems less likely as: (1) the shells had been largely

weathered prior to their use in the SRBR (therefore little organic matter was still attached to the shell) and, (2) the sulfate reduction results did not support this hypothesis as little and inconsistent sulfate removal differences were observed between the systems containing mussel shell or limestone.

Overall, it seems that the reactors operating on a long HRT_n were truly reduced systems operating at equilibrium and dominated by a low ORP environment and higher sulfate reduction rates. On the other hand, the reactors operating on a shorter HRT_n were constantly being shifted from equilibrium due to the continuous addition of oxygenated MIW and were therefore dominated by a higher ORP environment, and resulted in lower sulfate reduction rates.

The organic waste materials seemed to have provided enough short-term carbon sources for the SRBR to efficiently initiate the MIW treatment and to sustain it during the first 5-month period. Although the SRBR (except system LS-S) efficiently treated the MIW until the end of the second 5-month period, the higher C/N ratios reported for the bark and the bark mulch (Chapter 3) may have resulted in a relatively poor biodegradability of the substrate. Over the long-term, this could result in low concentrations of dissolved simple organic compounds, and would therefore halt the treatment. As the compost was used as a source of microorganisms (i.e. the inoculum), it is likely that the bacteria were not adapted to the extreme conditions encountered in the SRBR, and therefore might have suffered from the low pH and the high metal concentrations associated with MIW. This probably led to a sub-optimal bacterial establishment and possibly explains the low sulfate removal rates measured in the present study.

Finally, metal removal mechanisms are thought to have included sulfide, (oxy)hydroxide, and carbonate minerals precipitation, as well as adsorption onto the organic matter (especially for Mn). In the following Chapter (Chapter 5), chemical and mineralogical analyses were performed on the SRBR's spent substrates and PHREEQC modeling was used in comparison with the chemical and mineralogical analyses. Further analysis of the results from this chapter (especially the metal removal data) is provided after consideration of these other sources of information. Together, they will help in understanding the removal mechanisms occurring in each SRBR system.

CHAPTER 5

POST-TREATMENT AUTOPSIES: INVESTIGATING METAL REMOVAL MECHANISMS

5.1 Introduction

The objectives of this chapter were (1) to characterize the substrate mixtures and the individual materials used in the SRBR post-treatment (i.e. the spent substrates), and (2) to investigate the metal removal mechanisms taking place in SRBR using a complex organic mixture.

A number of chemical and mineralogical analyses were used to address these objectives. These included a sequential extraction procedure (SEP) determining the partitioning of metals within the spent substrate mixtures, a separate adsorption experiment to probe the capacity of the organic materials to scavenge Mn and Zn, as well as X-ray diffraction (XRD) and scanning-electron microscopy (SEM-EDS) measurements to identify solid mineral phases present within the spent substrates. Finally, chemical data from Chapter 4 was used in the modeling program PHREEQC to compute saturation indices of minerals potentially precipitating within the SRBR and compared with the SEP results. The SEP was conducted in the Environmental Laboratory of the Civil and Natural Resources Engineering Department at the University of Canterbury, and the SEM-EDS microanalyses were performed in the Mechanical Engineering Department at the University of Canterbury. The sorption experiment was done at CRL Energy Ltd. Laboratory in Christchurch, and the XRD analyses were performed by CRL staff in their Environmental Laboratory in Lower Hutt, New Zealand.

This chapter presents the results from the above-mentioned analyses and discusses the metal removal occurring within the SRBR. Additionally, a brief literature review of SEP that supplements the metal removal section of Chapter 2 is presented below.

5.1 Metal removal mechanisms and analytical techniques

Although the general processes responsible for the treatment of MIW in SRBR are now relatively well understood, the exact metal removal mechanisms, and especially the environmental and design factors affecting them, still remain unclear. Due to the numerous and inherently complex processes responsible for metal removal in SRBR and more generally in passive treatment systems, previous studies do not always agree. For example, even though metal-sulfide precipitation is the targeted metal removal mechanism, other processes such as filtration, complexation, cation exchange, adsorption, (oxy)hydroxide, oxide, and carbonate precipitation can also occur. Furthermore, the metal removal processes change over time, and depend on several factors including the reactor design (e.g. flow design, organic substrate composition, microbial community, hydraulic retention time) and the MIW chemistry (Gibert et al. 2002; Gusek 2005; Neculita et al. 2007a; Sobolewski 1999). For example, while adsorption is an important removal process upon the start-up of a new engineered SRBR, sulfide precipitation only takes place later, once the SRB are acclimated and the reducing conditions conducive to sulfate reduction are established (Machemer and Wildeman 1992; Neculita et al. 2008b). Furthermore, while some metals are relatively easy to remove from MIW (e.g. Al, Fe), other metals and metalloids (e.g. Mn, As, Se) can be more recalcitrant and harder to immobilize.

SEP have been widely used to study the distribution and the speciation of metals in soils, sediments, and waste materials (Hass and Fine 2010). SEP is a wet chemistry methodology that selectively targets metal species associated with specific and operationally defined solid-phases (e.g. water soluble, exchangeable, etc.). During the extraction procedure the samples are exposed to a sequence of chemicals, with each successive step becoming more aggressive and/or more specific to a defined mineralogical phase. Assuming that each step is more aggressive than the previous one, SEP can also be used to gain information on the metal's bioavailability and mobility (Hass and Fine 2010; Rao et al. 2007). For example, the metals extracted during the first step (i.e. the water soluble fraction) are more bioavailable than the metals associated with the last step (i.e. the residual fraction).

A large number of SEP have been established and tested, and because these procedures can use slightly different chemical reagents and concentrations, and/or different operational parameters (i.e. temperature, reaction time), the results from such studies can greatly vary and direct comparisons can sometime be difficult to perform (Hullebusch et al. 2005). Moreover, a number of issues associated with SEP can limit the precision and the exactitude of the methodology. For example issues associated with the limited selectivity of the reagents (i.e. the precision of the reagent to attack only one mineral phase and leave the others untouched), the possibility of metal re-distribution onto the remaining residues (i.e. once extracted, the metals can potentially re-adsorb onto the remaining residues), and the insufficiency of a reagent if the metal concentrations are too high (i.e. if not enough reagent is used, then not all the metals will be extracted) have been reported. It is also crucial to remember that the mineral phases extracted during the SEP are only operationally defined mineral fractions and one should not associate

these fractions with real existing mineral phases without considerable caution and sound geochemical explanations. Nevertheless, when carefully performed and taking these limitations into account, SEP can help understanding the metal removal mechanisms occurring in SRBR and other passive treatment systems, as well as the metal mobilization and biological availability (Dundar et al. 2011; Lo and Yang 1998). More importantly, the strength of such a methodology lies in its ability to compare samples. In other words, when a single and well-defined methodology is used in a study, the possibility to easily compare different samples from a single system and/or from different systems allows researchers to gain knowledge on microenvironments importance and development, as well as spatial and temporal variability of the metal removal mechanisms.

A brief description of the most common mineral phases targeted in SEP is presented below. For detailed information on SEP, readers are referred to Abollino et al. (2006), Hass and Fine (2010) and Rao et al. (2007). Most SEP are divided into six fractions:

(1) **Water soluble:** free and weakly complexed ions, and soluble salts. The metals associated with this first phase are the most labile and available to the biota;

(2) **Exchangeable:** weakly adsorbed ions (non-specifically sorbed). Metals can be released by small changes in the pH or the ionic strength of the surrounding solution (e.g. the action of environmentally abundant cations such as Ca, K or Mg can easily displace these metals) (Abollino et al. 2006);

(3) **Acid extractable:** bound to carbonates and specifically sorbed ions. This fraction is an important sink for metals (especially when Fe-Mn oxides are scarce). This phase is very sensitive to small changes in pH (Rao et al. 2007);

(4) **Reducible:** iron and manganese oxyhydroxides. These minerals can scavenge various trace metals through mechanisms such as co-precipitation, surface complexation, and sorption (Cornell and Schwertmann 2004; Post 1999), but are considered thermodynamically unstable under anoxic and reducing conditions (Tessier et al. 1979). This means that metals sorbed in aerobic environments can be remobilized when the oxyhydroxides dissolve in reducing conditions.

In addition, based on the findings of Piatak et al. (2006; 2007), who studied the mineralogy of mine waste and stream sediments both in the USA and in New Zealand, it was hypothesized that hydroxysulfate minerals (e.g. jarosite) would only be partially removed during the 4^{th} extraction step, and that well crystalized hydroxysulfates would mainly be dissolved during the last extraction step (i.e. the residual fraction).

(5) **Oxidisable:** bound to organic matter and/or sulfide minerals. Living and dead microorganisms as well as organic detritus can accumulate trace metals through bioaccumulation and complexation with humic and fulvic acids. Thus, destroying the organic materials (e.g. through chemical or biological oxidation) will resolubilize metals sorbed onto or incorporated into organics. Similarly, strong oxidizing conditions will dissolve metal sulfides;

(6) **Residual metals:** contained within a mineral lattice and or within silicates. Metals associated with this last fraction are not expected to be released under normal environmental conditions. In the present case, the residual fraction is assumed to include well crystalized (oxy)hydroxides, as

well as metals originally contained within the limestone or the mussel shell fragments (silicates will not be dissolved).

Mineralogical analyses such as X-ray diffraction (XRD) and scanning electron microscopy (SEM) have also frequently been used to identify solid-phase minerals in MIW treatment systems. However, due to the poor crystallinity of the precipitates, their low abundance with regards to the organic materials, a low detection limit, and the high heterogeneity of the samples, only a few studies have been able to identify trace metal precipitates through XRD analyses (Herbert et al. 1998; Sobolewski 1999; Swash and Monhemius 2005). On the other hand, SEM equipped with an energy dispersive spectrometer (EDS) has proven to be a much more successful technique (Gibert et al. 2005; Herbert et al. 2000; Neculita et al. 2008b).

5.2 Materials and Methods

5.2.1 Chemical analyses and samples collection

All chemical reagents used were analytical grade, and all glassware was previously acid-washed in 10 % HNO_3 for a minimum of 24 h, and rinsed three times with tap water and three times with deionized water. Metal analyses were performed via ICP-MS (Agilent, 7500cx), according to Standard Methods 3125B (APHA 2005). Quality assurance and quality control was achieved through the use of accredited standards, blanks, and replicated analyses. Duplicate analyses of total solids (TS) and volatile solids (VS) were performed on wet unground samples (Method 2540 G, APHA, 2005) to measure the moisture content and to obtain an indication of the organic

content. TS and VS measurements were repeated until variation between duplicates was < 5 %. Both SEP and adsorption studies were conducted at room temperature (23-25°C).

After ten months of operation, the influent MIW was stopped and the reactors were opened and drained under a high flow of nitrogen gas (BOC, 99.99 % purity). Spent substrate samples were manually retrieved using a plastic hand trowel from both the top and the bottom of the reactors (approximately 5 to 7 cm from the top and bottom sections of the reactive mixture). Composite samples from each of the four systems were collected and immediately placed in nitrogen-purged double-zip bags and frozen (-18 °C). While still frozen, approximately 200 g of samples were ground to < 1 mm size fraction using an electric mill. The ground samples were used for the SEP described below.

5.2.2 Sequential extraction procedure

The sequential extraction procedure used in this study is based on the methodology established by Jong and Parry (2004b). It is designed to separate metals into the six operationally defined fractions as presented above. Figure 5-1 provides an overview of the SEP and the detailed methodology is presented below. The present methodology deviates from the original method from Jong and Parry (2004b) for the extraction of fractions 3 and 6. Because the acid extractable step (fraction 3) can also removes metals specifically sorbed on Mn-oxides (e.g. Mn-oxides are very sensitive to pH changes around 5), the pH of the sodium acetate extraction solution was adjusted to 5.5 instead of 5 (Hass and Fine 2010). The residual fraction (fraction 6) was obtained using the APHA method 3030F (hot HNO_3-HCl acid digestion) instead of the hot perchloric acid

digestion used by Jong and Parry (2004b). Figure 7-3 (Appendix B) show some pictures of the experimental set-up.

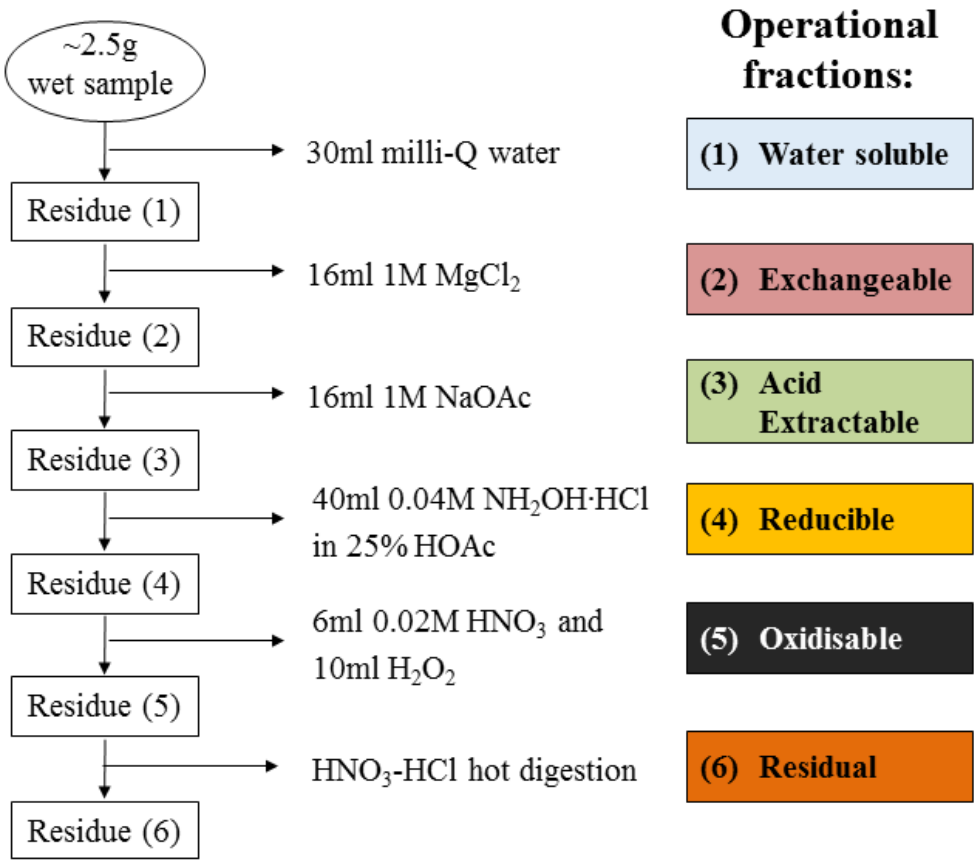


Figure 5-1. Sequential extraction procedure overview (adapted from Jong and Parry (2004b).

(1) Water soluble species: Approximately 50g of frozen ground sample was placed in a nitrogen-filled glove bag overnight at room temperature in order to thaw it. Then 2.5g (± 0.01g) of wet sample material was weighed in a 50 ml centrifuge tube and extracted using 30 ml of mili-Q water, and mechanically agitated using a rotary shaker for 2h. The speed of the rotary shaker was set so that the mixture was constantly maintained in suspension.

(2) Exchangeable ions: The residue from (1) was extracted with 16 ml of 1M $MgCl_2$ adjusted to pH 7 and mechanically agitated for 1 h.

(3) Acid extractable (bound to carbonates): The residue from (2) was treated with 16 ml of 1M NaOAc (sodium acetate) adjusted to pH 5.5 with HOAc (acetic acid) and mechanically agitated for 5 h.

(4) Reducible (bound to Fe-Mn oxides): The residue from (3) was extracted with 40 ml of 0.04M $NH_2OH{\cdot}HCl$ (hydroxylamine hydroxide) in 25 % (v/v) HOAc and mechanically agitated for 4 h, then heated at 96°C in a water bath for 1 h with occasional manual agitation (approx. every 15 min).

(5) Oxidisable (bound to organic matter and/or sulfides): The residue from (4) was digested for 1 h at room temperature with 6 ml of 0.02M HNO_3 and 10 ml of 30 % H_2O_2 (adjusted at pH 2 with concentrated HNO_3). Then it was heated at 85°C in a water bath for 2 h, before adding an additional 6 ml of 30 % H_2O_2 (pH 2) and the content digested for another 3 h at 85°C. Occasional manual agitation (approx. every 15 min) was performed during the entire 5^{th} step. After cooling down to room temperature, 10 ml of 3.2M NH_4OAc (ammonium acetate) in 20 % (v/v) HNO_3 was added, the sample further diluted to 40 ml with milli-Q water, and agitated for 30 min. The NH_4OAc step was performed to prevent re-adsorption of extracted metals onto the oxidized sample residue.

(6) Residual fraction: The residue from (5) was carefully transferred into Pyrex 200 ml conical beakers using milli-Q water. The solution was then digested following APHA method 3030F (total HNO_3-HCl hot acid digestion). Briefly, 5 ml of concentrated HNO_3 (Fisher, analytical grade 69 %) was added to the solution and gently heated on a hot plate, carefully avoiding boiling. Concentrated nitric acid was added and the solution heated until the digestate was light

in color and stopped changing color with additional digestion. Then 10 ml of concentrated HCl (Fisher, analytical grade 36 %) was added and the solution heated for an additional 30 min. The final solution was filtered using Whatman paper n°541 and the filtrate made up to 100 ml with milli-Q water.

Between each step of the extraction, the solid-liquid samples were centrifuged at 5000 rpm (Hermle Z383) for 15 to 20 min. The supernatant was then carefully transferred in a centrifuge tube, filtered at 0.45 µm using a syringe filter, acidified to pH < 2 with concentrated nitric acid (Fisher, analytical grade 69 %), and stored at 4°C until metal analyses via ICP-MS. Before proceeding to the next extraction step, the solid residue was washed with 16 ml of deionized water and mechanically agitated for 10 min. The rinses were separated, filtered, and retained for analysis following the above mentioned procedure. For quality control purposes, each spent substrate sample was analyzed in triplicate to ensure the reproducibility of the methodology and triplicate pseudo-total digestions of each sample were performed following the same HNO_3-HCl hot acid digestion as used for the residual fraction. A single blank containing all the reagents, but no spent substrate samples was also analyzed for each fraction. The results from the blank analysis were later subtracted from the actual spent substrate sample results. Pseudo-total results were compared with the sum of each extracted fraction using Equation 5.1 (Hullebusch et al. 2005), where the concentrations were in mg of metal per kg of dry material.

$$\text{Recovery (\%)} = (\text{sum of each fraction} / \text{pseudo-total}) \times 100 \qquad (5.1)$$

In order to minimize oxidation and help preserve the metal speciation, the SEP experiment was conducted in a 500 L glove-bag (Sigma-Aldrich) filled with high purity nitrogen (BOC, 99.99 % purity). When taken outside of the glove bag (for centrifugation, shaking or storage purposes), the samples were kept in tightly closed 50 ml centrifuge tubes wrapped with parafilm. Additionally, all extractant reagents were previously purged with high purity N_2 for a minimum of 30 min, and all disposable materials (including the centrifuge tubes) were kept in the glove-bag for a minimum of 24 h prior to using them.

5.2.3 Adsorption edge experiment

An adsorption batch experiment was performed to evaluate the organic mixture's ability to retain Mn and Zn. The organic mixture was microwaved for 3 min at 1000 W to eliminate bacterial activity, ground and sieved to obtain a particle size < 1 mm, and oven-dried at 55°C overnight to remove all moisture. The adsorption edge experiment was conducted over a pH range of 3 to 9 following the methodology from Bibby and Webster-Brown (2006). A "parent" solution of 0.1M $NaNO_3$ was spiked with metal salts ($Mn(NO_3)_2$ and $Zn(NO_3)_2$) to obtain final concentrations of 5 mg/L Mn and 2 mg/L Zn. These concentrations and a solid to metal ratio of 1 g organic material per 100 ml of metal solutions were chosen to approximate 10 pore-water volumes when compared to the bioreactor flow-through experiment. 500 ml bottles containing the solid/liquid mix were kept overnight at 4°C to allow equilibrium and complete re-hydration of the solid surfaces. The solid/liquid slurry solutions were then carefully transferred in 2 L beakers and continuously agitated using a magnetic stirrer. The solution pH was lowered to 3 using diluted HNO_3 and then slowly raised to pH 9 by incremental addition of diluted NaOH. 12 ml aliquots were taken at approximately each 0.5 pH unit increment and placed into 50 ml centrifuge tubes

and immediately placed on a rotary shaker. After a 24 h period of constant agitation, the samples were centrifuged, the supernatant final pH verified, and 5 ml samples were filtered through 0.45 μm syringe nylon filters, acidified to pH < 2 with concentrated HNO_3 and analyzed for metal concentrations. Two control experiments were carried out to determine (1) the degree of metal removal through adsorption onto the containers walls and possible precipitation of metal (oxy)hydroxides and oxides (control #1); and (2) the release of metals from the organic mixture (control #2). Control experiment #1 was conducted without the organic mixture, but with the spiked metal solutions, while control experiment #2 was performed with the organic mixture but without adding the metal solutions.

5.2.4 X-ray diffraction

Mineralogical composition of the spent substrates was determined by X-ray diffraction (XRD) by CRL Energy Ltd. staff (Lower Hutt, New Zealand). The spent substrate mixture samples were dried at 110°C overnight and immediately ground and prepared as unoriented powder mounts. The diffractometer (Philips PW1729) was equipped with a graphite monochromator and a Co anode X-ray source. Analyses were performed from 5 to 80° (2θ) at 1° and 1 min/step scanning time. Phase identification and quantification were carried out by a Siroquant search and match program.

5.2.5 Scanning electron microscopy

Scanning electron microscopy (SEM) microanalyses were performed on a JEOL JSM6100 microscope equipped with an Oxford Aztec silicon drift detector energy-dispersive spectrometer

(EDS), at the University of Canterbury, Mechanical Engineering Department. Samples were oven-dried overnight at 105° prior to being manually disaggregated and mounted on plastic stubs and coated in approximately 25 nm of carbon. Bark, bark mulch, mussel shells and limestone subsamples were looked at individually, and both the inner and outer sides of the mussel shell samples were analyzed. The morphological observations were performed at an accelerating voltage of 20 kV.

5.2.6 Geochemical modeling

The PHREEQC model was used to calculate the SI of selected mineral phases potentially precipitating in the SRBR substrates and later compared with the SEP results. Input parameters used in the model were as follows: metals and sulfate from influent concentrations; ORP, D.O. and temperature from pore-water measurements; alkalinity and pH from effluent measurements (results from Chapter 4), and chosen to best evaluate the SRBR's potential to precipitate metals within the substrate reactive mixtures.

5.3 Results and Discussion

5.3.1 Sequential extraction results

The SEP results are shown in Figure 5-2, Figure 5-3, Figure 5-4, and Figure 5-5. Table 5-1 shows absolute and relative values for top and bottom sections of each system. The coefficient of variation of triplicate analyses for fractions 1 to 5 was found to be ≤ 15% for all samples (n = 288), except for 11 samples in fraction 1 (coefficient of variation ranged between 15.8 and 23.7 %), 4 samples in fraction 2 (15.9 - 32.9 %), 3 samples in fraction 3 (16.7 - 22.2 %), and 1 sample in fraction 4 (21.8 %). Coefficient of variation in fraction 5 was constantly < 10 %. Overall, these results indicated that the methodology and the precision of the analytical and laboratory work were satisfactory. Additionally, two important points are to be noted: (1) the residual step (fraction 6) had a much larger coefficient of variation (usually between 20 to 30%, but up to 85.2 % in one case); (2) the metal losses during rinses performed in between each extraction step were substantial when compared to the total (i.e. extract plus rinse), with values constantly between 10 - 58 % for fractions 1 and 2 (water soluble species and exchangeable ions) and between 11 - 51 % for fractions 3 to 5. Based on this observation, the extracts and the rinses have been aggregated and considered together for the overall interpretation of the results (e.g. the rinse between the 2nd and the 3rd extraction step was aggregated with the results obtained for the 2nd step and so on).

The blank results (Table 5-2) were overall small when compared to the metal extracted from the spent substrate samples. For Fe and Al, the higher values were found in fractions 1, 2 and 3 and ranged between 2.8 - 11.2 %, and 5.6 - 33.3 % for Fe and Al, respectively (all other values for Fe and Al were < 1 %). Considering that these fractions (i.e. water soluble, exchangeable and

carbonate-bound) cumulatively contained < 1 % of the total extracted amount of Fe and Al, these findings had little implication for the interpretation of the final results. For Mn, the blank results were much smaller in all fractions and in all samples (blanks constantly contained ≤ 0.7% of the metals extracted from the corresponding fraction in the spent substrate samples). For Zn, the blank values ranged between 1.3 - 31.0 % in fractions 1, 2 and 6 (all other values for Zn were < 5 %). For Ni and Cu, the blank values were constantly higher. For Ni, these values ranged between 8.9 and 41.5 % in fractions 1 and 2 and between 0.5 and 89.4 % in fractions 3, 4, 5 and 6. For Cu, they ranged between 1.5 and 58.2 % in fractions 1 and 2, and between 0.9 and 102.9 % in fractions 3, 4, 5 and 6. The higher values obtained for Ni, Cu and Zn were constantly found in fractions 1 and 2 and can partially be explained by the overall smaller metal contents (thus, the higher relative errors) and by the chemical nature of the extractant reagents themselves. For example, fraction 1 was extracted and rinsed using the same reagent (i.e. milli-Q water).

The metal recoveries (%) in the SEP, calculated following Equation 5.1 are shown in Table 5-3. The higher values obtained for the sum of each extracted fraction compared to the pseudo-total values is a common issue associated with SEP and can be explained by several factors including: contamination of the reagents, low concentrations of some metals in the spent substrate samples (resulting in higher relative errors), precision of the laboratory work, and redistribution of the metals into the remaining phases once a targeted phase has been extracted. In the present case, the lower values were obtained for Mn across all four systems. As Mn was associated with relatively readily dissolvable mineral phases (see figures and discussion below), the hot acid digestion method used for the pseudo-total analyses easily dissolved these samples and therefore recovered most of the Mn. On the other hand, the higher recovery values were constantly found

for the limestone containing systems. Considering that limestone pieces originally contained more metals than the mussel shell fragments (see discussion in Chapter 3, section 3.4.2), it is hypothesized that the pseudo-total digestions were not conducted for long enough, thus a full digestion of the samples might not have been achieved.

The VS measurements used to approximate the organic matter content in the substrates after 10 months of MIW treatment indicated that the reactive mixtures containing mussel shell had 26.2 to 43 % of organic matter, while the mixtures containing limestone had only 25.7 to 33.5 %. Although this methodology has clear limitations and probably slightly overestimates the true organic content (i.e. the mass loss during combustion also includes some mineral salts present in the spent substrates), it still provided a useful indication for the interpretation of the SEP results, especially for the organic matter/sulfide fraction.

Table 5-1. Sequential extraction results showing metal partitioning in the spent substrates. Results are expressed as mean of triplicate analyses and reported in mg/kg of dry material or in percentage (%). F1 (water soluble), F2 (exchangeable ions), F3 (acid extractable), F4 (reducible), F5 (oxidisable), F6 (residual). Reagent blank values are already subtracted from the spent substrate sample values. Pseudo-totals (%) indicate recovery values (%). MS-S and MS-L indicate mussel shell systems short and long HRT; LS-S and LS-L indicate limestone systems short and long HRT, respectively.

SRBR	Sample location	Fractions	Al (mg/kg)	Al (%)	Fe (mg/kg)	Fe (%)	Mn (mg/kg)	Mn (%)
MS-S	top	F1	1.91	0.03	0.86	0.02	21.98	3.5
		F2	2.30	0.03	4.52	0.10	247.69	39.1
		F3	0.62	0.01	19.97	0.42	189.95	30.0
		F4	290.49	4.3	553.47	11.8	116.23	18.4
		F5	943.33	14.1	1308.94	27.8	19.06	3.0
		F6	5470.26	81.5	2814.17	59.9	38.44	6.1
		Sum	*6708.91*	*100*	*4701.94*	*100*	*633.35*	*100.0*
		Pseudo-tot.	*4204.10*	*159.6*	*3189.31*	*147.4*	*784*	*80.8*
	bottom	F1	2.62	0.03	1.79	0.03	18.43	3.1
		F2	1.73	0.02	4.92	0.09	163.32	27.3
		F3	0.62	0.01	19.22	0.36	199.79	33.4
		F4	271.53	3.2	719.09	13.6	141.24	23.6
		F5	1338.27	15.9	1740.09	33.0	31.77	5.3
		F6	6787.12	80.8	2787.01	52.9	43.43	7.3
		Sum	*8401.89*	*100*	*5272.12*	*100*	*597.98*	*100.0*
		Pseudo-tot.	*4760.16*	*176.5*	*3757.29*	*140.3*	*444*	*134.8*
MS-L	top	F1	1.09	0.03	1.12	0.03	19.75	4.5
		F2	1.05	0.02	4.39	0.13	113.11	25.9
		F3	0.56	0.01	20.37	0.61	174.21	39.9
		F4	316.58	7.3	742.20	22.4	96.49	22.1
		F5	818.12	18.8	1205.33	36.4	16.00	3.7
		F6	3206.46	73.8	1338.88	40.4	16.58	3.8

		Sum	*4343.85*	*100*	*3312.28*	*100*	*436.13*	*100.0*
		Pseudo-tot.	*3201.68*	*135.7*	*3581.48*	*92.5*	*578*	*75.4*
	bottom	F1	1.25	0.02	1.43	0.03	21.89	4.1
		F2	1.67	0.02	6.70	0.14	184.20	34.1
		F3	0.67	0.01	27.50	0.56	173.55	32.1
		F4	301.95	4.2	753.78	15.3	112.09	20.7
		F5	1172.68	16.3	1630.41	33.2	18.86	3.5
		F6	5726.84	79.5	2493.72	50.8	29.69	5.5
		Sum	*7205.07*	*100*	*4913.53*	*100*	*540.28*	*100.0*
		Pseudo-tot.	*3809.95*	*189.1*	*3729.29*	*131.8*	*526*	*102.7*
LS-S	top	F1	1.92	0.01	1.26	0.01	16.08	2.1
		F2	1.63	0.01	2.98	0.01	174.13	23.1
		F3	1.18	0.00	17.45	0.08	185.84	24.7
		F4	359.48	1.3	859.29	3.8	231.64	30.8
		F5	1719.58	6.4	2527.17	11.1	34.25	4.6
		F6	24701.36	92.2	19310.07	85.0	110.27	14.7
		Sum	*26785.16*	*100*	*22718.22*	*100*	*752.22*	*100.0*
		Pseudo-tot.	*10364.35*	*258.4*	*8501.44*	*267.2*	*734*	*102.5*
	bottom	F1	5.93	0.02	2.71	0.01	13.16	2.4
		F2	3.82	0.01	3.41	0.01	127.03	23.2
		F3	1.46	0.00	15.80	0.07	98.93	18.1
		F4	495.43	1.3	820.24	3.4	96.16	17.6
		F5	1741.81	4.7	1924.81	8.0	22.28	4.1
		F6	35156.85	94.0	21194.43	88.5	190.03	34.7
		Sum	*37405.30*	*100*	*23961.40*	*100*	*547.59*	*100.0*
		Pseudo-tot.	*12317.29*	*303.7*	*7749.79*	*309.2*	*352*	*155.5*
LS-L	top	F1	4.37	0.02	1.47	0.01	13.80	3.2
		F2	0.46	0.00	2.45	0.02	84.63	19.4
		F3	0.48	0.00	13.70	0.09	104.93	24.0
		F4	351.15	1.8	921.88	5.8	123.76	28.3
		F5	1763.47	9.1	2384.35	15.0	26.09	6.0

	F6	17238.72	89.0	12521.74	79.0	83.81	19.2
	Sum	*19358.66*	*100*	*15845.59*	*100*	*437.03*	*100.0*
	Pseudo-tot.	*18537.51*	*104.4*	*5441.75*	*291.2*	*488*	*89.6*
bottom	F1	4.77	0.02	1.52	0.01	9.94	2.1
	F2	0.52	0.00	2.69	0.02	88.58	18.9
	F3	0.64	0.00	12.92	0.08	82.29	17.5
	F4	364.07	1.5	944.92	5.6	142.55	30.4
	F5	1871.70	7.8	2453.11	14.6	29.89	6.4
	F6	21631.27	90.6	13348.35	79.6	115.72	24.7
	Sum	23872.98	100	16763.52	100	468.98	100.0
	Pseudo-tot.	9348.24	*255.4*	4989.65	*336.0*	461.2	*101.7*

Table 5-1 (cont.) Results for Ni, Cu, Zn.

SRBR	Sample location	Fractions	Ni (mg/kg)	Ni (%)	Cu (mg/kg)	Cu (%)	Zn (mg/kg)	Zn (%)
MS-S	top	F1	4.99	11.8	0.85	2.7	0.92	0.4
		F2	3.89	9.2	0.51	1.6	1.09	0.5
		F3	2.17	5.1	0.24	0.8	8.65	4.0
		F4	4.80	11.3	0.20	0.6	128.80	60.1
		F5	13.96	33.0	19.58	62.4	46.67	21.8
		F6	12.55	29.6	10.00	31.9	28.10	13.1
		Sum	*42.34*	*100.0*	*31.38*	*100.0*	*214.23*	*100.0*
		Pseudo-tot.	*18*	*235.1*	*12.56*	*249.8*	*165.6*	*129.3*
	bottom	F1	7.01	18.8	0.24	0.7	0.49	0.2
		F2	2.57	6.9	0.14	0.4	0.35	0.2
		F3	1.31	3.5	0.14	0.4	2.69	1.4
		F4	3.98	10.7	0.32	1.0	113.23	58.0
		F5	12.91	34.7	16.95	51.1	50.38	25.8
		F6	9.46	25.4	15.40	46.4	28.20	14.4

		Sum	*37.25*	*100.0*	*33.18*	*100.0*	*195.34*	*100.0*
		Pseudo-tot.	*17*	*224.7*	*14.13*	*234.8*	*120.1*	*162.6*
MS-L	top	F1	0.20	0.4	0.20	0.9	0.67	0.3
		F2	0.64	1.4	0.13	0.6	1.95	0.9
		F3	1.39	3.1	0.21	1.0	22.39	10.1
		F4	7.75	17.5	0.24	1.1	153.49	69.6
		F5	23.83	53.7	14.56	67.1	28.73	13.0
		F6	10.54	23.8	6.35	29.3	13.38	6.1
		Sum	*44.35*	*100.0*	*21.70*	*100.0*	*220.59*	*100.0*
		Pseudo-tot.	*41*	*107.7*	*13.71*	*158.3*	*125.3*	*176.1*
	bottom	F1	0.14	0.4	1.35	4.4	0.66	0.3
		F2	0.50	1.6	0.18	0.6	2.94	1.2
		F3	0.85	2.7	0.30	1.0	22.81	9.1
		F4	5.24	16.8	0.36	1.2	162.39	64.7
		F5	19.89	63.7	19.59	64.3	41.15	16.4
		F6	4.63	14.8	8.69	28.5	20.88	8.3
		Sum	*31.23*	*100.0*	*30.47*	*100.0*	*250.83*	*100.0*
		Pseudo-tot.	*20*	*154.5*	*13.36*	*228.0*	*105.4*	*237.9*
LS-S	top	F1	1.07	1.0	1.95	2.2	10.33	2.5
		F2	1.22	1.2	0.58	0.6	4.03	1.0
		F3	0.81	0.8	0.24	0.3	18.73	4.5
		F4	6.98	6.7	0.97	1.1	125.79	30.4
		F5	30.78	29.7	21.22	23.5	38.07	9.2
		F6	62.64	60.5	65.40	72.4	217.27	52.5
		Sum	*103.50*	*100.0*	*90.37*	*100.0*	*414.23*	*100.0*
		Pseudo-tot.	*39*	*266.6*	*35.82*	*252.3*	*231.2*	*179.2*
	bottom	F1	1.27	1.0	4.32	4.0	4.41	1.0
		F2	1.24	0.9	2.22	2.0	3.71	0.8
		F3	0.82	0.6	1.01	0.9	21.82	4.8
		F4	6.65	5.0	1.21	1.1	173.37	38.2
		F5	27.05	20.2	28.49	26.2	78.87	17.4

		F6	96.62	72.3	71.48	65.7	171.37	37.8
		Sum	*133.65*	*100.0*	*108.73*	*100.0*	*453.55*	*100.0*
		Pseudo-tot.	*36*	*372.2*	*74.60*	*145.7*	*217.8*	*208.3*
LS-L	top	F1	0.13	0.2	1.35	2.9	2.72	1.4
		F2	0.15	0.3	0.69	1.5	1.54	0.8
		F3	1.50	2.8	0.21	0.5	6.25	3.2
		F4	2.29	4.2	0.72	1.6	71.23	36.4
		F5	14.70	27.2	13.34	29.1	23.29	11.9
		F6	35.31	65.3	29.52	64.4	90.45	46.3
		Sum	*54.07*	*100.0*	*45.82*	*100.0*	*195.48*	*100.0*
		Pseudo-tot.	*21.8*	*248.0*	*41.71*	*109.9*	*86*	*227.3*
	bottom	F1	0.15	0.2	3.49	6.5	2.99	0.7
		F2	0.18	0.3	1.62	3.0	1.45	0.3
		F3	0.36	0.6	0.24	0.5	3.25	0.8
		F4	3.31	5.1	1.07	2.0	85.62	20.5
		F5	20.78	32.2	14.51	27.2	41.64	9.9
		F6	39.67	61.5	32.52	60.8	283.62	67.8
		Sum	64.46	100.0	53.46	100.0	418.56	100.0
		Pseudo-tot.	26.6	*242.7*	30.06	*177.9*	*236.0*	*177.3*

Table 5-2. Reagent blank values in mg/L.

Fractions	Al	Fe	Mn	Ni	Cu	Zn
F1	0.36	0.08	0.02	0.53	0.12	0.14
F2	0.13	0.33	0.59	0.35	0.03	0.11
F3	0.11	0.77	0.20	0.33	0.09	0.10
F4	2.37	4.35	0.39	0.82	0.20	1.32
F5	1.88	0.71	0.08	0.39	0.26	1.16
F6	10.32	4.28	0.09	0.49	1.81	2.20
Sum	*15.17*	*10.53*	*1.37*	*2.91*	*2.51*	*5.02*

Table 5-3. Metal recoveries (%) from the SEP. Top and bottom samples are considered together.

	Al	Fe	Mn	Ni	Cu	Zn
MS-S	168.0	143.9	107.8	229.9	242.3	145.9
MS-L	162.4	112.1	89.1	131.1	193.1	207.0
LS-S	281.1	288.2	129.0	319.4	199.0	253.0
LS-L	179.9	313.6	95.6	152.6	143.9	308.7

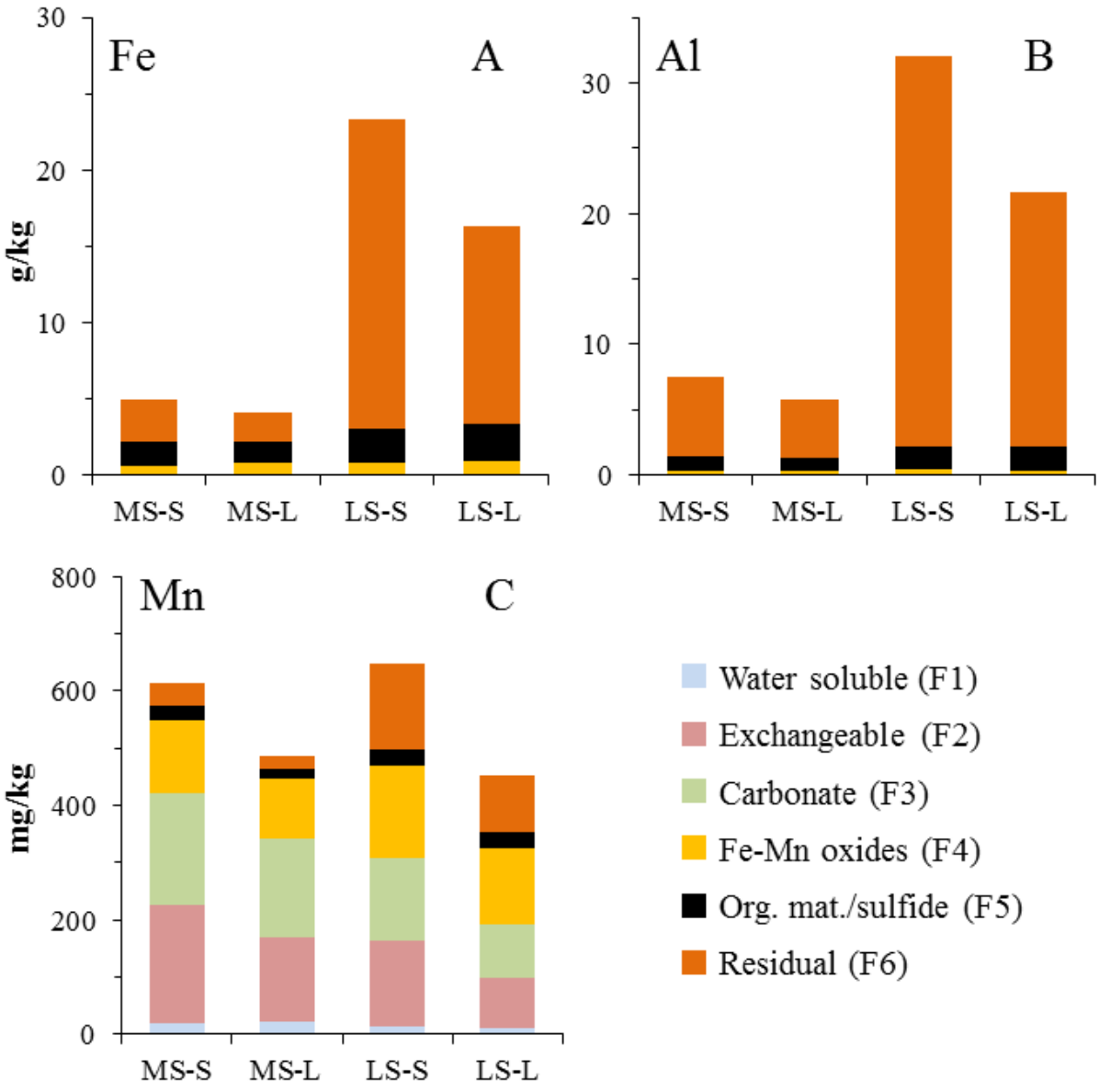


Figure 5-2. SEP results showing total concentration and partitioning of (A) Fe, (B) Al, and (C) Mn in the spent reactive substrates. Results are average of top and bottom samples, and are reported in g/kg of dry materials (Fe, Al) and in mg/kg of dry materials (Mn). MS-S and MS-L indicate mussel shell short and long HRT; LS-S and LS-L indicate limestone systems short and long HRT.

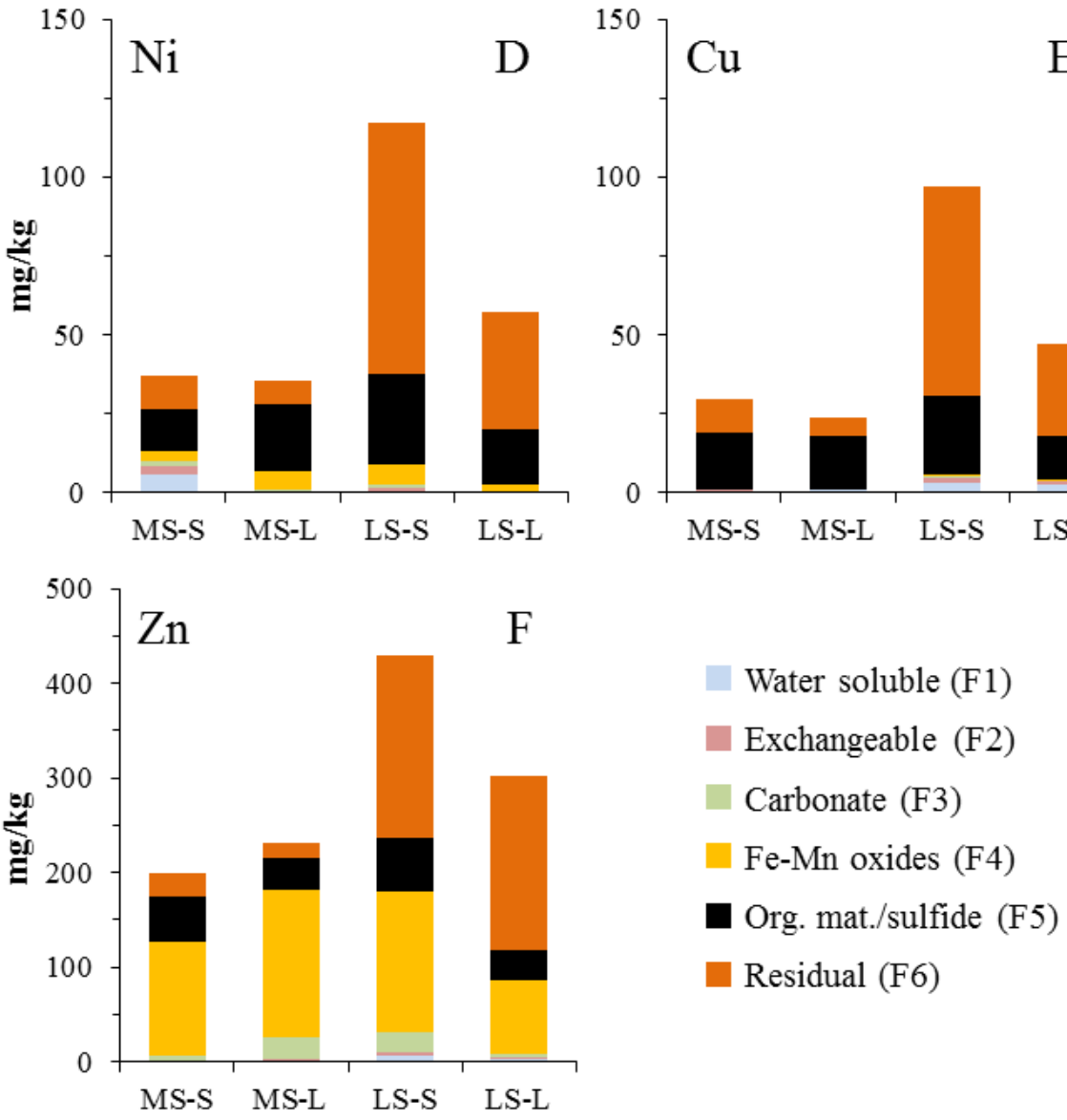


Figure 5-3. SEP results showing total concentration and partitioning of (D) Ni, (E) Cu, and (F) Zn in the spent reactive substrates. Results are average of top and bottom samples, and are reported in mg/kg of dry materials. MS-S and MS-L indicate mussel shell short and long HRT; L LS-S and LS-L indicate limestone systems short and long HRT.

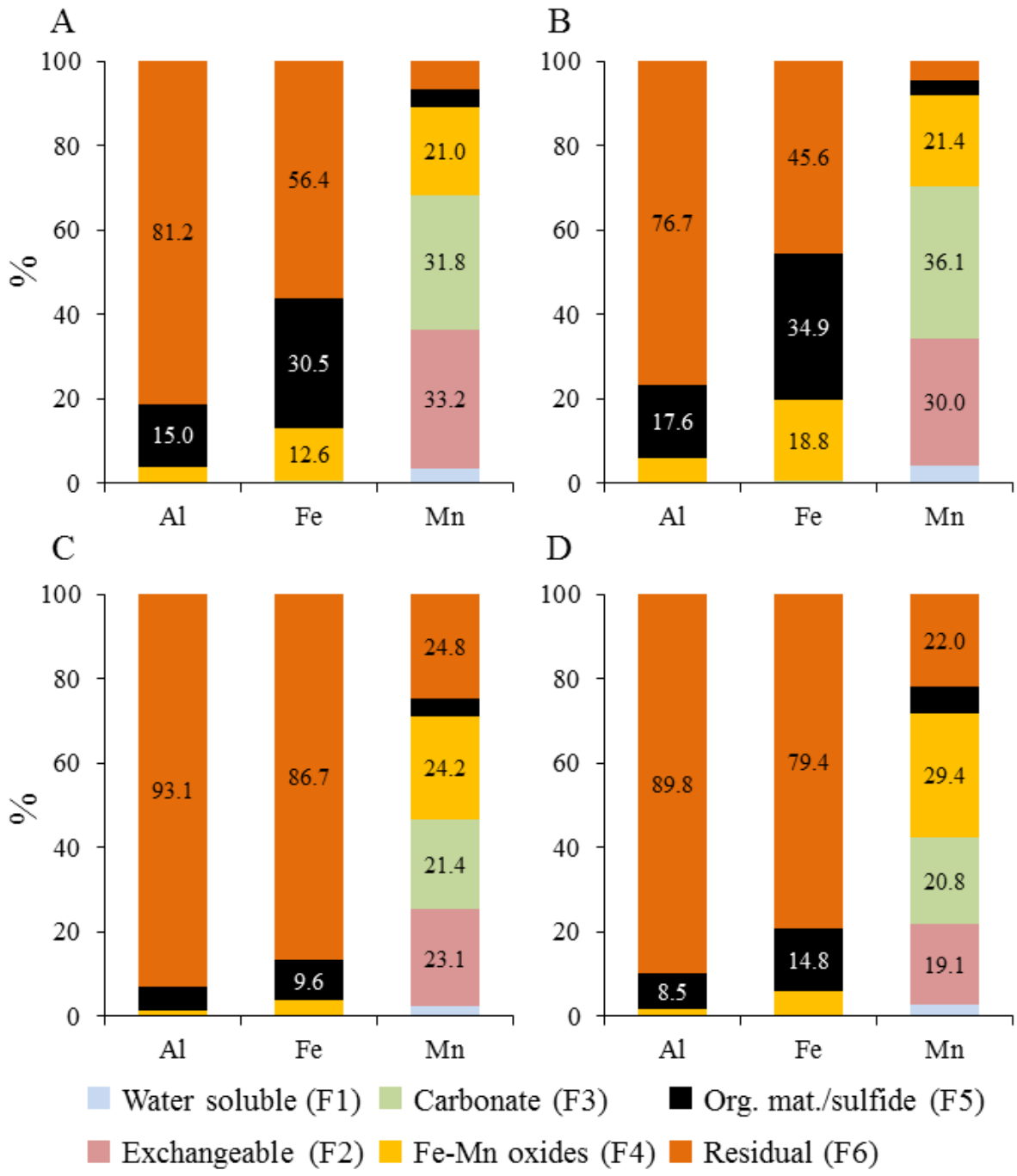


Figure 5-4. SEP results showing relative percentages (%) of Al, Fe and Mn in (A) MS-S, (B) MS-L, (C) LS-S, and (D) LS-L systems Results are average of top and bottom samples.

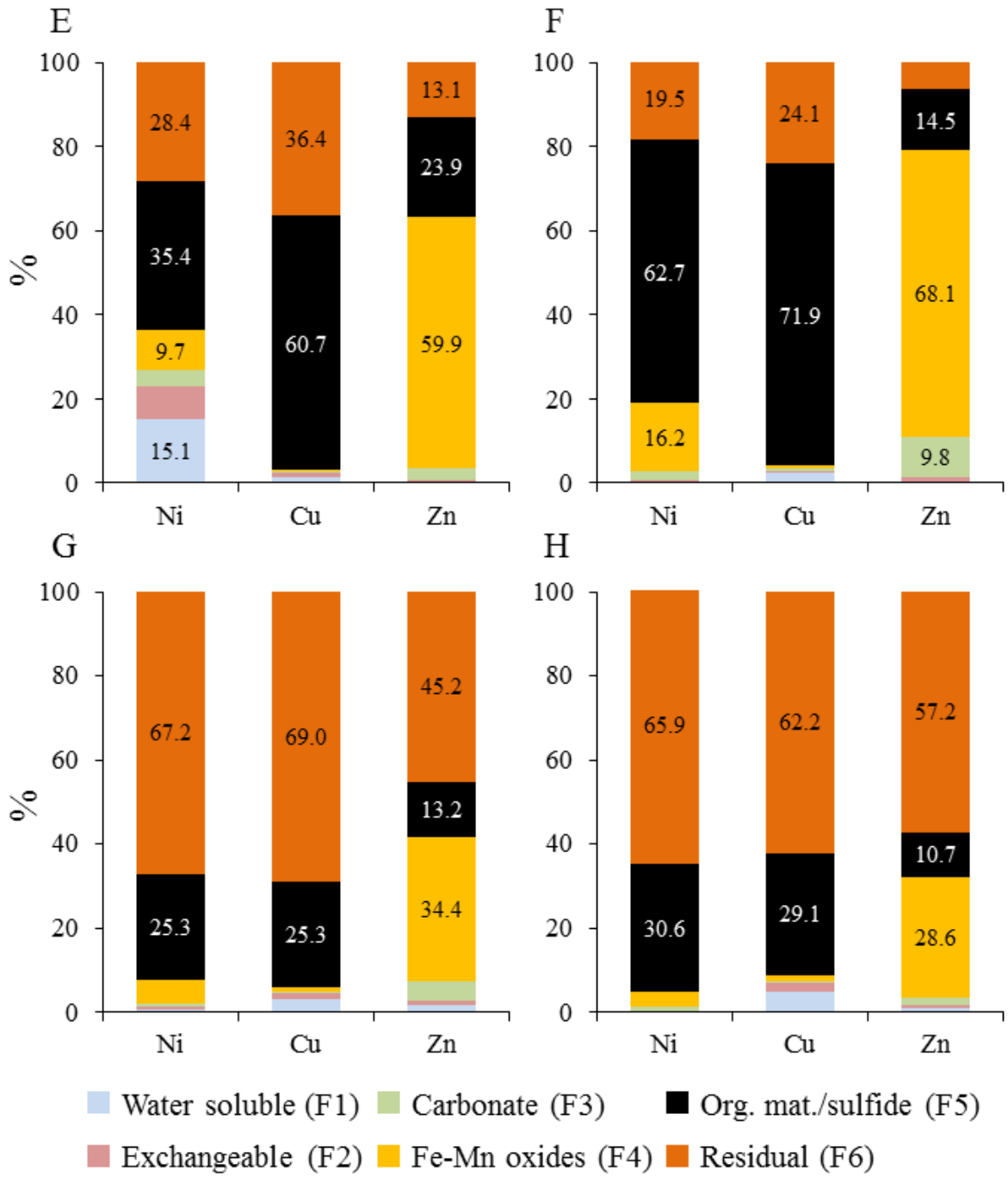


Figure 5-5. SEP results showing relative percentages (%) of Ni, Cu and Zn in (E) MS-S, (F) MS-L, (G) LS-S, and (H) LS-L systems Results are average of top and bottom samples.

Based on the sum of total extracted heavy metals in the SEP, the metal concentrations followed the same order in each system for both the top and bottom sections (Al > Fe >> Mn > Zn > Ni > Cu), which was almost identical to the median influent metal concentrations (Fe > Al > Mn > Zn > Ni > Cu). Furthermore, based on the total amount of metal found in each fraction, several observations can be made: Mn was always the most abundant metal in fractions 1, 2, and 3, followed by Ni, Zn (in F1), Ni and Fe (in F2), and Fe and Zn (in F3). Metal partitioning in fractions 4, 5 and 6 was very similar in each system and followed the general order of Fe > Al >> Zn > Mn > Ni ≥ Cu. Figure 5-2 and Figure 5-3 show a greater amount of metal retained in the residual fraction in LS systems compared to MS systems. This observation simply shows that the limestone pieces, prior to being used in the SRBR, contained more metal than the mussel shells fragments (as shown in Table 3-6), and do not reflect an additional metal removal from the MIW. Assuming that each gram of spent substrate contained 0.6 g of limestone or 0.5 g of mussel shells, it was estimated that each kilogram of spent substrate originally contained approximately 2.52 and 0.38 g of Fe, 1.66 and 0.39 g of Al and 0.13 and 0.014 g of Mn for the limestone and the mussel shells mixtures respectively (based on results presented in Table 3-3 and Table 3-6). Additionally, short HRT systems show more accumulated metals than long HRT systems, which reflect the fact that short HRT systems treated approximately 3 times more MIW compared to the long HRT reactors from a mass balance perspective (i.e. the short HRT reactors received about 3 times the amount of MIW compared to the long HRT systems over the course of the 10 months flow-through experiment, see Chapter 4). Specific findings for each metal are presented and discussed below.

Iron:

Figure 5-2 shows a greater amount of Fe retained in the residual fraction in limestone systems compared to mussel shell systems. Again this observation shows that the limestone pieces prior to being used for MIW treatment contained approximately 5.5 times more Fe than the mussel shells fragments, and do not reflect an additional Fe removal from the MIW. Considering the VS results and the geochemical behavior of iron, it is hypothesized that most Fe recovered from fraction 5 (organic matter/sulfide) was associated with sulfide minerals rather than with the organic matter. Regardless of the fact that the limestone originally contained more iron (supposedly in fraction 6), Fe was found to be mainly associated with the residual and sulfide fractions in all four systems. More Fe was associated with the sulfide fraction in MS systems (up to 35 %) compared to LS systems (10-15 %). As Fe(III) is suspected to be removed as oxyhydroxide and Fe(II) as sulfides, it is likely that more Fe(II) was available in MS systems to react with the biogenic sulfides, and more Fe(III) was present in LS systems and removed as oxyhydroxides. This hypothesis is supported by the lower ORP values reported for MS reactors and the similar sulfate percentage removal reported for MS and LS systems (Chapter 4). Furthermore, the longer HRT systems resulted in additional Fe being removed as sulfides, when compared to the short HRT; an observation which is again consistent with the higher sulfate reduction and the lower ORP values reported for systems MS-L and LS-L. It was also hypothesized that the small amount of Fe recovered from fraction 4 (Fe-Mn oxides) in all four systems was due to the localized precipitation of (oxy)hydroxides in oxidized microenvironments, and in the bottom gravel layers where the in-situ conditions were still aerobic and oxidizing (see Figure 7-2).

Aluminum:

Al was predominantly found in the residual fraction (≥ 76 %) in all four systems, with an additional 15 to 18 % (MS systems), and 5 to 8 % (LS systems) present in the organic matter/sulfides fraction. Because the SEP did not have a specific step or fraction targeting Al, its presence in fraction 6 suggested both fresh and old, but rather well crystalized oxide, (oxy)hydroxide and hydroxysulfate minerals. These findings are in accordance with previous studies (Nordstrom and Ball 1986; Zagury et al. 1997). Indeed, in passive treatment systems like SRBR, Al is assumed to be removed as an amorphous hydroxide (gibbsite-like) and/or as a hydroxysulfate mineral (basaluminite-like) (Adams and Rawajfih 1977; Nordstrom 1982; Watzlaf et al. 2004). Additionally, because the precipitation of $Al(OH)_{3(am)}$ is almost an instantaneous process once the pH is raised above 4.5 (Hedin et al. 1994), we found more Al retained in the bottom sections compared to the top sections of the SRBRs (pH measured at the pore-water sampling port situated at reactor half-height was constantly > 5 apart for reactor LS-S). The small amount of Al found in fraction 5 is possibly explained by adsorption of Al-(oxy)hydroxides on organic matter (i.e. aluminol groups can bound to organic matter through ligand exchange).

Manganese:

In MS systems, Mn was principally found in the exchangeable (30-33 %), the carbonate (32-36 %) and the Fe-Mn oxides fractions (21 %). In LS systems, Mn was associated in similar relative concentrations (approx. 20-30 %) with the exchangeable, carbonate bound and reducible fractions. The removal of Mn through carbonate mineral precipitation (e.g. rhodochrosite) is supported by the fact that more Mn was associated with carbonates in the

MS reactors compared to the LS systems, which is in accordance with the higher alkalinity concentrations measured in MS systems. Additionally, more Mn was removed with the carbonates in the top parts of short-HRT reactors for both alkalinity sources (Table 5-1), an observation that further supports the removal of Mn as rhodochrosite (i.e. pH, alkalinity and Ca concentrations were higher in the top sections of the reactors compared to the bottom sections). It is also likely that the organic matter associated with the mussel shell (i.e. mussel flesh remains, and chitin-related proteins associated with the shells) provided additional adsorption sites, possibly explaining the greater proportion of Mn associated with the exchangeable fraction in MS systems compared to LS systems. It is also possible that higher-pH microenvironments close to the mussel shell or the limestone fragments allowed for the precipitation of Mn-oxides (e.g. birnessite), possibly explaining the 21 %, and 24-29 % of Mn recovered with fraction 4 in MS and LS systems, respectively. The removal of Mn through carbonate precipitation and adsorption has been recognized by others studies (Dufresne et al. 2015; Robinson-Lora and Brennan 2010b; Swash and Monhemius 2005; Waybrant et al. 1998).

Zinc:

Most Zn in the MS reactors was associated with the Fe-Mn oxides (60-68%), and organic matter/sulfides fractions (14-24 %), while only 4-13 % was found in the residual fraction. In LS reactors, Zn was mostly found in the residual fraction (45-57 %), the Fe-Mn oxide fraction (28-34 %) and only 11-13 % in the organic matter/sulfides phases. Although limestone originally contained approximately 2.1 times more Zn in the limestone compared to the mussel shell (Table 3-6), these results suggested that Zn was removed through adsorption onto and/or

co-precipitation with Fe-oxides, as well as sulfide precipitation in both MS and LS systems. These findings are in agreement with other SEP studies which found most Zn to be associated with sulfides and Fe-Mn oxides (Jong and Parry 2004b; Neculita et al. 2008b). Additionally, Wallman et al. (1993) showed that zinc sulfides can dissolve in acidified reagents at pH 5 (e.g. due to the greater solubility of ZnS compared to other sulfide minerals, Table 2-3). This implies that sulfide minerals, especially ZnS, are dissolved in several extraction steps and not only in the oxidisable fraction, potentially leading to an underestimation of the true zinc sulfide amount. Finally, system MS-L (where the alkalinity concentration was the highest) also retained about 10 % of Zn in fraction 3, supposedly through carbonate precipitation.

Nickel:

More than 80 % of Ni was found with the oxidisable and the residual fractions in systems MS-L, LS-S and LS-L, while only about 64 % was associated with the same fractions in MS-S reactor. In MS systems, most Ni was retained as sulfides (oxidisable fraction) with a clear influence of the HRT; the longer HRT resulting in more Ni removed as sulfides. Additionally, some Ni was retained with the water soluble and the exchangeable fractions (in MS-S) and with the reducible fraction (in MS-L). These results are in contrast with other studies which found Ni to be associated with carbonates (almost no Ni was recovered from fraction 3 in the present study). Indeed Jong and Parry (2004b) found Ni to be mostly associated with the carbonate fraction, and Neculita et al. (2008b) recovered Ni in the water soluble, the exchangeable and the carbonate fractions. Although the amount is relatively small, it seems that a short HRT resulted in more Ni associated with the water soluble and the exchangeable

fractions. Similarly to Zn, NiS could have dissolved not only in the oxidization step (fraction 5), but also in the previous acid extraction steps (fractions 3 and 4, Hullebusch et al. 2005).

Copper:

More than 90% of Cu was retained with the oxidisable and the residual fractions in all four systems. These results are in agreement with most previous studies (Lo and Yang 1998; Machemer and Wildeman 1992; Neculita et al. 2008b; Waller and Pickering 1992). Indeed, because of its low solubility CuS is one of the first metal sulfide to precipitate, and Hullebusch et al. (2005) showed that Cu was more refractory to the H_2O_2 oxidation step than Ni or Zn. Additionally, Cu displays a strong affinity for organic matter with which it forms strong and stable complexes with humic acids, further supporting its presence in fraction 5.

In summary, apart from Mn which was mostly associated with the exchangeable and the carbonate fractions, and Zn which was essentially recovered from the Fe-Mn oxides fraction, all other metals were recovered from the organic matter/sulfide and the residual fractions. Fe and Al, the two most abundant metals in the influent MIW cumulatively accounted for less than 1 % in fractions 1, 2, 3, and more than 80 % of Fe and 96 % of Al were contained in fractions 5 and 6. These findings indicate that the immediate mobility and bioavailability of both Fe and Al are considered very low. In contrast, Mn, the third most abundant metal in the influent MIW, was found to be much more labile, with > 68 % and > 42 % recovered from fractions 1, 2, and 3 in MS and LS systems, respectively. These findings indicate that Mn

leaching could become problematic if the environmental conditions (i.e. pH and ORP) of the spent substrate were to change.

In addition, long HRT systems retained more Fe, Ni and Cu sulfides (supported by the lower ORP and the higher sulfate reduction values), and more Mn as carbonates which is in accordance with the higher alkalinity values and the finding of Lebron and Suarez (1999) suggesting that an HRT of ≥ 3 days was necessary to precipitate rhodocrosite.

5.3.2 Adsorption edge experiment results

Figure 5-6 shows that both Mn and Zn were effectively adsorbed onto the organic mixture used in the SRBR. Figure 5-7 shows the results from control experiment #2 (i.e. release of metals from the organic mixture). A Table in Appendix A-5 shows the initial and final pH (after 24 h of agitation) measured during the adsorption study.

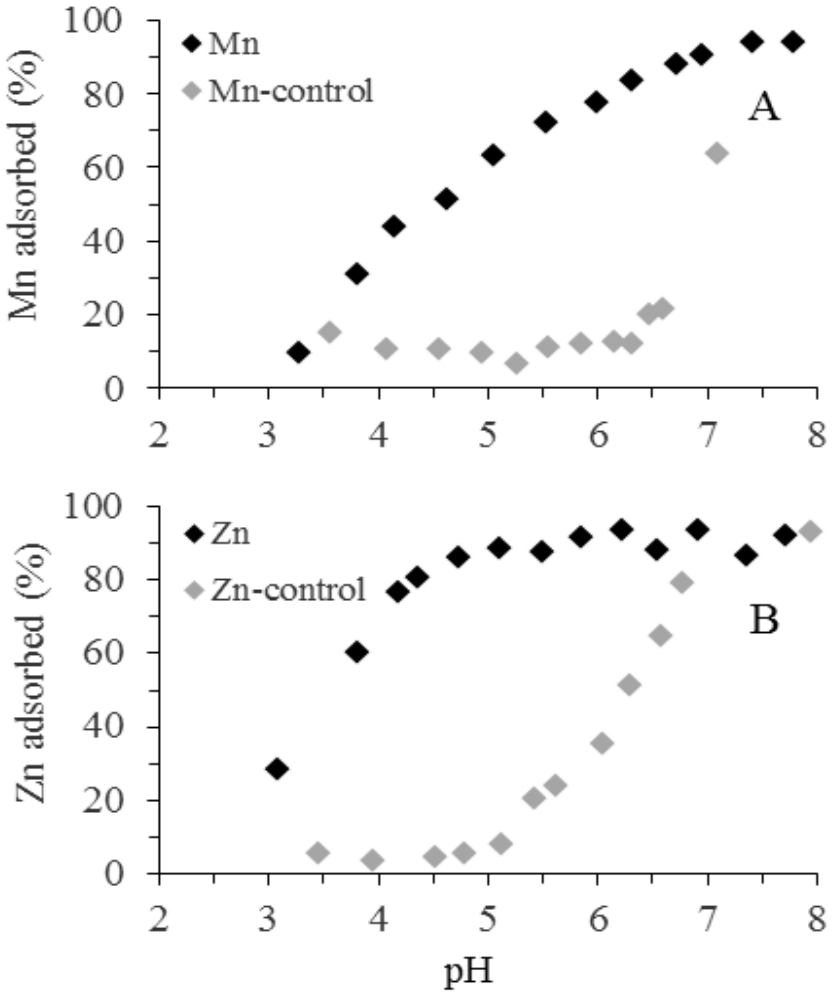


Figure 5-6. Adsorption of (A) Mn and (B) Zn onto the organic materials (initial concentrations of 5 mg/L Mn and 2 mg/L Zn, and a suspended substrate concentration of 10 g/L). Control experiments did not have substrate present.

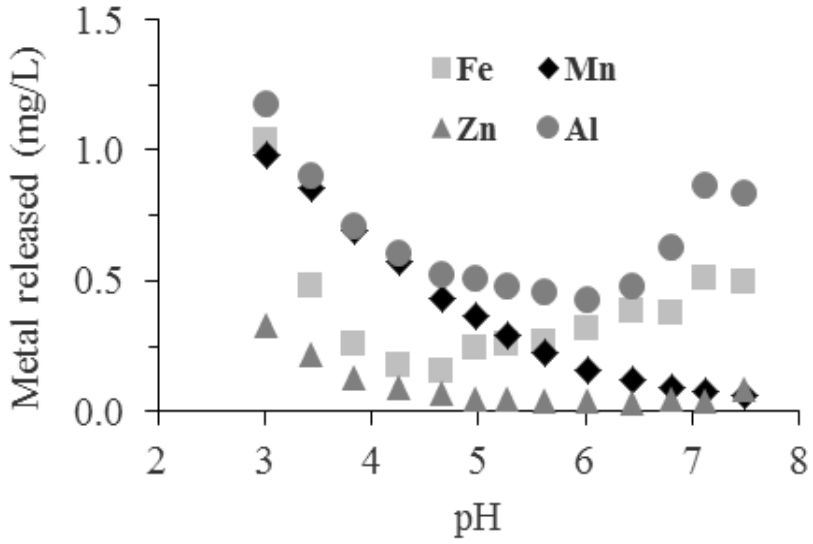


Figure 5-7. Metals released during the adsorption edge experiment (control experiment #2).

Mn adsorption increased linearly with increasing pH, and at pH 7 up to 70 % of dissolved Mn was adsorbed. Even though no clear adsorption edge was visible, an approximate pH_{50} value of 4.5 can be derived. From the Mn-control experiment it was found that up to pH 6.5 less than 20 % Mn was removed through adsorption onto the container walls or removed through precipitation (presumably as a manganese oxide), and only when the pH was raised above 7 were substantial amounts (63 %) of Mn removed via these mechanisms. Considering that during the flow-through SRBR experiment the effluent pH was constantly between 6 and 7.2, we can assume that adsorption was an important mechanism for Mn removal, at least during the start-up period. This hypothesis is supported by both the SEP results (i.e. substantial amounts of Mn were retained in the exchangeable fraction), and results from the previous chapter which showed that up to 80 % Mn was removed during the first few weeks of the flow-through experiment, followed by a constant decline (Figure 4-10). This was interpreted

as a consequence of adsorption sites available for metal binding declining with time. Similar adsorption behavior for Mn has been reported in MIW treatment systems (Gibert et al. 2005; Webster-Brown et al. 2012; Younger et al. 2002) and in natural river environments (Webster-Brown et al. 2012).

Zinc adsorption also increased with increasing pH, but a more distinct adsorption edge was visible, and the pH_{50} value was approximately 3.6. Between pH 3 and 5.5 almost 90 % of Zn was removed by adsorption; the Zn-control experiment showed > 20 % Zn removal only at pH > 5.5. Several studies have shown that Zn can adsorb onto organic matter, including living and dead biomass (Azabou et al. 2007; Gibert et al. 2005; Webster-Brown et al. 2012). Additionally, numerous studies have shown that Fe- and Mn-oxides can adsorb high concentrations of metals such as Zn, Cu, or Cd (Dyer et al. 2004; Jurjovec et al. 2002; Webster et al. 1998). Based on the SEP results and PHREEQC modeling, the adsorption of Zn onto Fe- and Mn-oxyhydroxides appears to dominate, rather than binding to the organic matter. Indeed, although Figure 5-7 show that little amount of metals were released by the organic materials during the adsorption experiment, the model PHREEQC indicated that at pH 4 and above, both Al- and Fe-(oxy)hydroxides started to precipitate.

5.3.3 Solids mineralogy

Mineral phases identified through XRD analyses are presented in Table 5-4. Because of the low concentration of metal precipitates, their poor crystallinity (e.g. Pósfai and Dunin-Borkowski (2006) showed that biogenic sulfides are characterized by a poorly-defined crystallinity), as well as the large heterogeneity of the samples, the analyses were unable to

identify many of the dominant mineral phases and no accurate mineral quantification was possible. A high background in the diffraction patterns indicated the presence of an amorphous fraction (i.e. organic materials and amorphous precipitates). Results presented in Table 5-4 are normalized to 100 % and do not include estimates of the amorphous components (only semi-quantitative estimates are provided).

Table 5-4. X-ray diffraction (XRD) results from the spent organic substrate mixture. Long and short HRT are considered together (samples from the top section of the reactors).

Samples	Phase Name	Phase Formula	Presence
MS	Aragonite	$CaCO_3$	Major
	Quartz	SiO_2	Minor
	Calcite	$CaCO_3$	Trace
	Albite	$(Na,Ca)(Si,Al)_3O_8$	Trace
LS	Calcite	$CaCO_3$	Major
	Quartz	SiO_2	Minor
	Albite	$(Na,Ca)Al(Si,Al)_3O_8$	Minor
	Illite	$(K,H_3O)(Al,Si)_4O_{10}(OH)_2$	Trace
	Pyrite	FeS_2	Trace

Aragonite (with trace of calcite) predominated in MS systems, but only calcite was identified in LS systems. This is in agreement with the XRD analyses performed on the alkalinity materials pre-treatment (Table 3-10) which showed that mussel shells comprised of > 96% of aragonite, the rest being identified as calcite, while limestone was made of pure calcite (> 99%). In addition, post-treatment XRD analyses performed on the alkaline materials alone showed that there was a preferential dissolution of the aragonite over the calcite in the mussel

shell samples (Figure 5-8). Indeed, post-treatment XRD analyses indicated that aragonite accounted for about 89 % of the total calcium carbonate, the remaining 11 % being calcite (compared to the pre-treatment results showing a mineral composition of approximately 96% aragonite and 4% calcite).

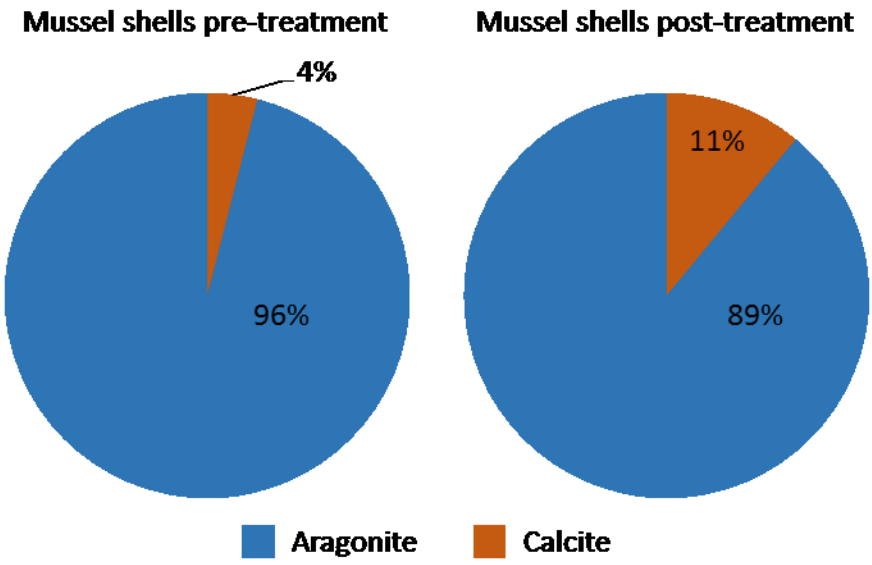


Figure 5-8. X-ray diffraction (XRD) results from the mussel shells fragments before and after the flow-through treatment (average of duplicate analyses).

Traces of albite and illite came from residual silicate minerals present in the compost used in the reactive mixture and did not precipitate during the experiments. Traces of an iron disulfide were detected (most likely pyrite, FeS_2) in one of the samples retrieved from reactor LS-L. Although Neculita et al. (2008b) found traces of mackinawite, greigite and goethite, and Herbert et al. (1998) detected what they described as a disordered mackinawite, most authors agree that XRD is not a suitable technique for the examination of spent organic substrates.

In contrast, SEM-EDS microanalyses (multiple localized scans with element mapping) performed on individual spent substrate materials (bark, bark mulch, mussel shell, and limestone) provided more useful information (Figure 5-9, Figure 5-10). Similar to the SEP results, SEM-EDS microanalyses showed higher Fe and Al concentrations than Mn, Zn or Cu concentrations, consistent with the substrate analyses and the influent MIW metal concentrations (Ni content was at the detection limit of 0.1 wt. % across all samples and therefore is not shown in Figure 5-9 and Figure 5-10). The EDS scans indicated more metal accumulation in bioreactors operating at a short HRT compared to the long HRT reactors (Figure 5-9 A, B, C, D, E). In contrast, the sulfur content on both the organic and the alkaline materials (Figure 5-9 F) showed that the longer HRT systems resulted in additional sulfur binding (presumably in the form of sulfides). These observations concurred with the water chemistry results, which showed that the longer HRT systems reduced more sulfate, but that the short HRT systems removed more metals from a mass balance perspective. Short HRT systems also showed more Fe accumulation than S (on all materials and in all systems, apart from bark in MS-S), suggesting that sulfide precipitation was not the only removal pathway for Fe. In contrast, long HRT reactors either displayed similar Fe and S contents or additional S compared to Fe, suggesting that precipitation of iron sulfide was an important removal mechanism in these systems.

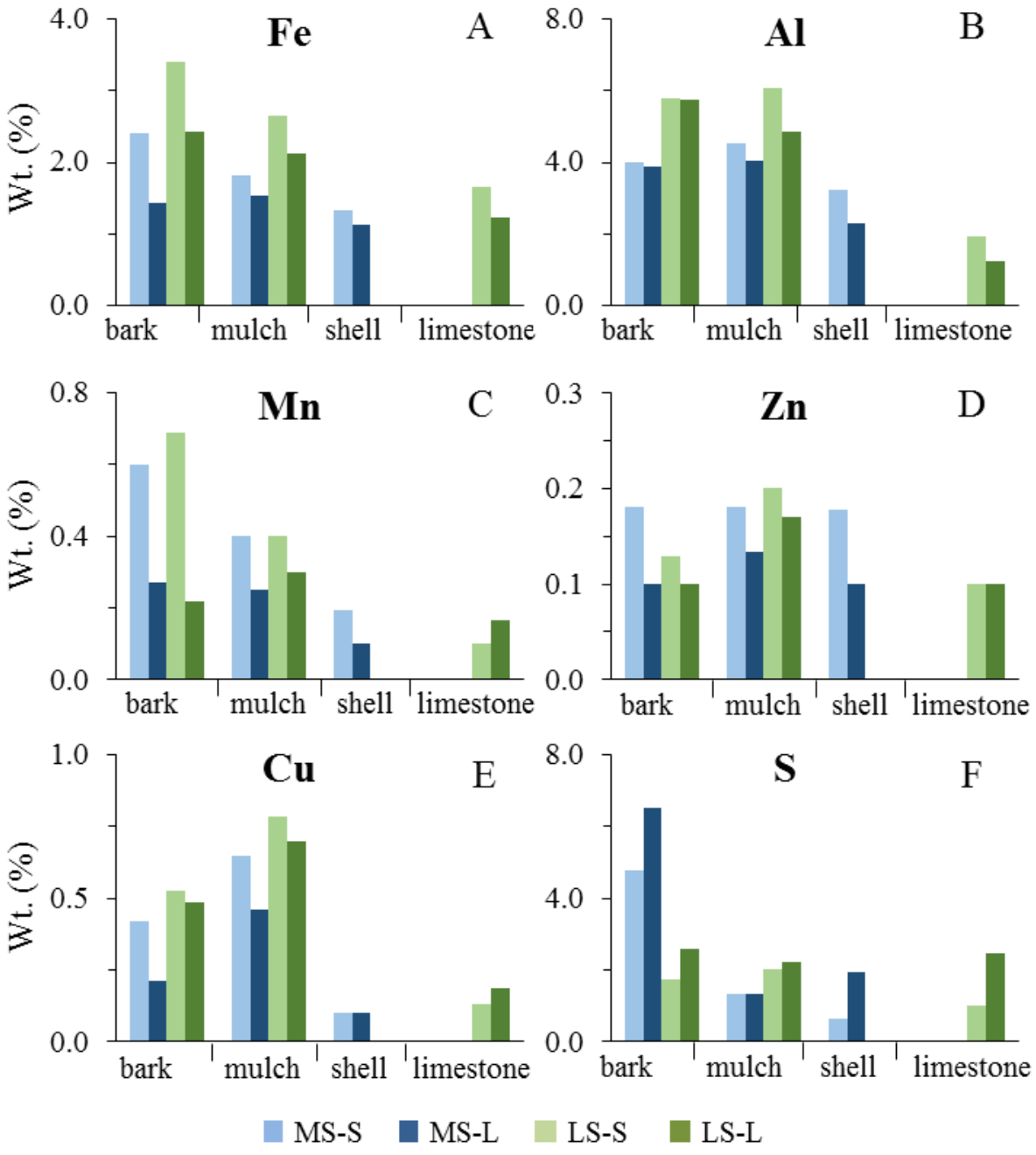


Figure 5-9. Average results from multiple EDS element mapping scans (n = 5 to 10) showing contents (wt. %) of (A) Fe, (B) Al, (C) Mn, (D) Zn, (E) Cu and (F) S on individual materials retrieved from the top parts of the reactors (short and long HRT samples are combined).

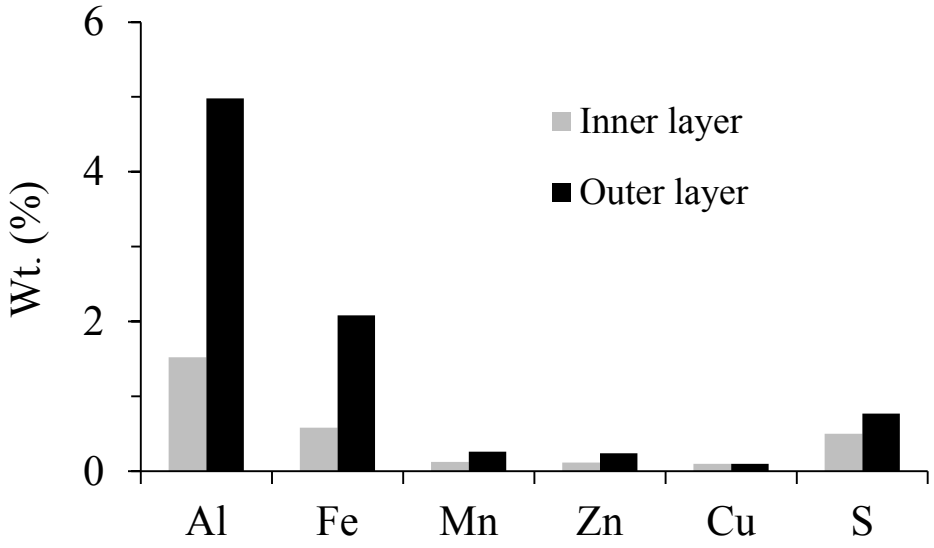


Figure 5-10. Average results from multiple EDS element mapping scans (n = 5) showing contents (wt. %) of Al, Fe, Mn, Zn, Cu and S on the inner and outer surfaces of mussel shells fragments retrieved from the top parts of the reactors, with short and long HRT sample combined.

Figure 5-10 showed more metals and more sulfur (presumably sulfides) retained on the outer side of the mussel shells compared to the inner side. The shells are comprised of three distinct layers: an inner shell layer (i.e. the inner side*, hypostracum*) primarily made of aragonite, a middle shell layer (*ostracum*) made of calcite and aragonite interbedded with proteins molecules, and an outer shell layer (i.e. the outer side, *periosctracum*) made of a nitrogen polysaccharide chitin (Cubillas et al. 2005). Therefore, considering that aragonite dissolves faster than calcite (Cubillas et al. 2005b), it is likely that the metals accumulating onto the inner side (i.e. aragonite layer) were continually released back in solution because of the faster dissolution. Additionally, the outer side (i.e. chitin layer) is likely to afford more adsorption sites compared to the inner aragonite layer, thus immobilizing more metals.

SEM-EDS also allowed for the clear identification of a discrete iron sulfide, and gypsum ($CaSO_4$) minerals (Figure 5-11). The average elemental composition of the iron sulfide mineral (wt. %) was 46 % S, and 38 % Fe (i.e. approximately a 1:2 molar ratio) suggesting pyrite (FeS_2). Similar spherical precipitates containing Fe and S have been reported by other studies (Gibert et al. 2003, 2005). Although the precipitation of $CaSO_4$ was not expected in the reducing conditions encountered within the SRBR substrates, its presence has previously been reported in the microenvironments within similar treatment systems (Rose et al. 2007).

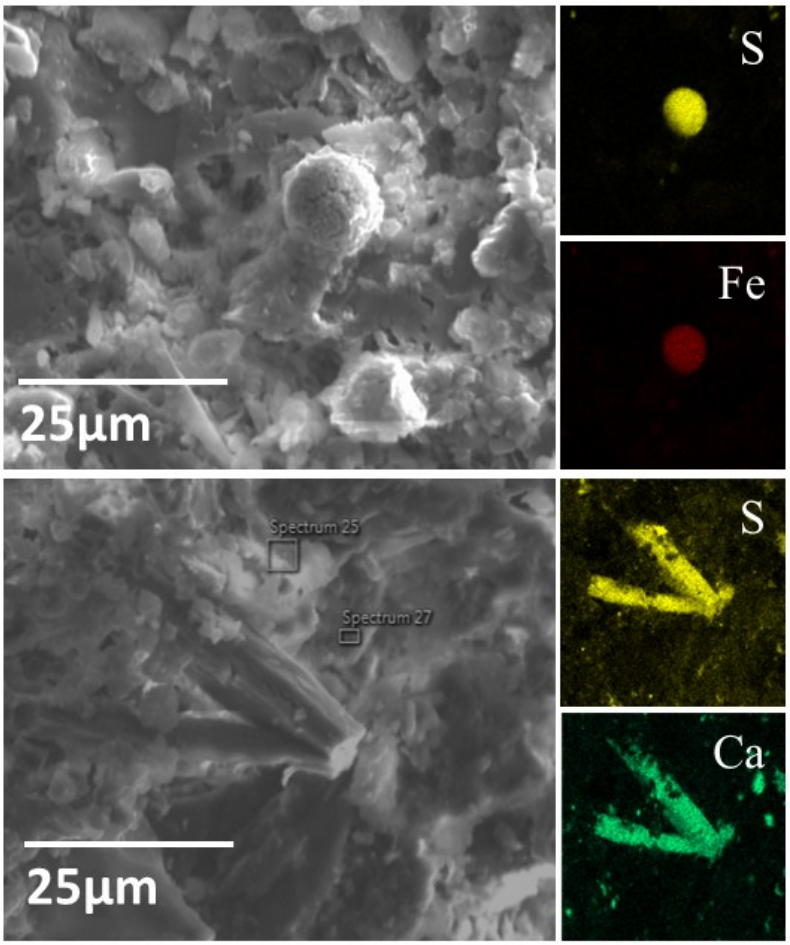


Figure 5-11. X-ray scans and element mapping obtained via SEM-EDS showing (A) an iron sulfide, (B) gypsum.

5.3.4 Geochemical modeling

A comparison of saturation indices (SI) predicted with PHREEQC for low temperature stable mineral phases, and inferred mineral phases suggested from the SEP experiment, is presented in Table 5-5. Note that the PHREEQC model did not take any kinetics implications into account and therefore the minerals reported by the model should be considered as indicative of the types of minerals that are likely to precipitate. A large number of similar but amorphous mineral phases, rather than well crystalized minerals, could have been included in the PHREEQC database and describe better the solids likely to form in these experiments. It is recognized that this PHREEQC modelling is meant to give indicative and suggestive results rather than definitive results about the mineral forms actually forming in the reactors. The model input parameters are shown in Appendix A-6 and the actual SI output values in Appendix A-7.

The geochemical model predicted the precipitation of low temperature stable iron (oxy)hydroxides (i.e. goethite and hematite), and sulfides (i.e. greigite, mackinawite, pyrite), and overall the SEP results mostly corroborated the model. While PHREEQC predicted (oxy)hydroxides to precipitate only in reactor MS-S (where the pore-water ORP values were higher), the SEP indicated that these minerals were found in both MS-S and MS-L. This difference is likely explained by the fact that aerobic microenvironments were also present in the MS-L system. On the other hand, pyrite was predicted in all systems (apart from MS-S) and greigite and mackinawite in both MS-L and LS-L, and their presence confirmed by SEP in all four systems. The negative SI values for Fe, Ni and Zn sulfides in system MS-S (CuS and Cu_2S were the only sulfide minerals showing positive SI values in system MS-S) were

supported by the higher ORP values reported for this system and likely explain by the ingress of O_2 through the pore-water sampling port.

Al geochemical removal was attributed to the precipitation of (oxy)hydroxides, hydroxysulfates, and oxides, an hypothesis supported by the model. For Mn, although PHREEQC suggested the precipitation of rhodochrosite only in system MS-L, the SEP results indicated that Mn carbonates likely precipitated in each of the four systems. Again, this difference is probably explained by the fact that the chemical inputs values were bulk geochemical parameters, and therefore precipitation in microenvironments close to the mussel shell or limestone fragments (where the pH would be higher), would not be predicted by the model. While PHREEQC predicted only zinc sulfides to precipitate (i.e. sphalerite and an amorphous ZnS phase), the results from the SEP suggested that Zn was also associated with the reducible fraction (Fe- and Mn-oxyhydroxides) and the carbonate fraction in system MS-L. Therefore, we can assume that both ZnS precipitation and additional removal through co-precipitation with and/or adsorption onto Fe-Mn oxides occurred. The model predictions for both Ni and Cu were mostly corroborated by the SEP results. Indeed PHREEQC predicted Ni to be removed through sulfide precipitation only, and Cu through sulfide precipitation mostly with additional removal as an oxide (CuO) in short HRT_n systems.

In addition, the model suggested the precipitation of an iron phosphate (i.e. vivianite) in the two short HRT systems, and Mn hydrogen phosphate (i.e. $MnHPO_4$) in all four reactors. Although, this hypothesis could not be verified via the SEP used in the present study (no extraction step specifically targeted phosphate minerals), others studies suggested the removal

of Mn through precipitation or chemisorption with phosphates (Robinson-Lora and Brennan 2010b; Carliell-Marquet and Wheatley 2002). In addition, the pore-water and effluent phosphate results discussed in Chapter 4 (Figure 4-3) further support this hypothesis. Finally, from the study by Hupfer et al. (2009), who studied the partitioning of phosphorus in calcite-rich lake sediments samples, and the study by Wang et al. (2013) who compared well-established SEP for the determination of P in sediments, it was hypothesized that the phosphate minerals (vivianite and $MnHPO_4$) were most likely to be retained within the residual phases. Indeed, these authors showed that mineral P was retained in the residual fraction after the samples were extracted using a solution of 0.5 M HCl (the last extraction step used in the present study used concentrated HCl and HNO_3 and should therefore have dissolved the P containing minerals).

Table 5-5. Mineral phases predicted by PHREEQC to precipitate in the SRBR and as inferred by the SEP. A "X" indicates that a SI > 0, and a shaded cell indicate if ≥ 9 % of the metal was associated with the corresponding mineral phase in the SEP.

	Mineral phases	Formula	MS-S	MS-L	LS-S	LS-L
Fe	Ferrihydrite	$Fe(OH)_3$	-	-	-	-
	Goethite	α-FeO(OH)	X	-	-	-
	Hematite	Fe_2O_3	X	-	X	-
	Vivianite	$Fe_3(PO_4)_2 \cdot H_2O$	X	-	X	-
	Siderite	$FeCO_3$	-	-	-	-
	Mackinawite	FeS	-	X	-	X
	Greigite	Fe_3S_4	-	X	-	X
	Pyrite	FeS_2	-	X	X	X
Al	Gibbsite	$Al(OH)_3$	X	X	X	X
	Al hydroxide	$Al(OH)_3$(am)	X	X	X	X
	Boehmite	AlOOH	X	X	X	X
	Basaluminite	$Al_4(OH)_{10}SO_4$	X	-	X	X
	Al oxide	Al_2O_3	X	X	X	X
Mn	Rhodochrosite	$MnCO_3$	-	X	-	-
	Mn phosphate	$MnHPO_4$	X	X	X	X
	Pyrolusite	MnO_2	-	-	-	-
	Manganite	MnO(OH)	-	-	-	-
Zn	Smithsonite	$ZnCO_3$	-	-	-	-
	Zincite	ZnO	-	-	-	-
	Sphalerite	ZnS	-	X	X	X
	Zn sulfide	ZnS (am)	-	X	X	X
Ni	Ni carbonate	$NiCO_3$	-	-	-	-
	Ni hydroxide	$Ni(OH)_2$	-	-	-	-
	Bunsenite	NiO	-	-	-	-
	Ni phosphate	$Ni_3(PO_4)_2$	-	-	-	-
	Ni sulfide	NiS (α,β,γ)	-	X	X	X
Cu	Cuprite	CuO_2	X	-	X	-
	Malachite	$Cu_2(OH)_2CO_3$	-	-	-	-
	Cu carbonate	$CuCO_3$	-	-	-	-
	Covellite	CuS	X	X	X	X
	Chalcopyrite	$CuFeS_2$	X	X	X	X
	Chalcocite	Cu_2S	X	X	X	X

5.4 Conclusions

The chemical and mineralogical analyses used to examine the spent substrates helped shed light on the metal removal processes occurring in SRBR using complex organic substrates to treat MIW. While metals were undoubtedly removed from solution, the exact processes that led to their removal can only be inferred indirectly. Overall, the present results indicated that SRBR successfully retained high concentrations of metals within the substrates, and suggested that metals were mostly retained in a non-mobile and non-bioavailable form. Although sulfide mineral precipitation clearly seems to have occurred, a great deal of indirect evidence indicates that other removal mechanisms took place including adsorption onto organic matter, and precipitation as carbonates, and (oxy)hydroxide minerals.

From the SEP study, it was established that all metals (except Mn) were mostly retained in fractions that require a chemical or a biological conversion process before being considered bioavailable (i.e. retained as (oxy)hydroxide, sulfide, and oxide minerals), thus their availability and mobility could be considered low. Mn, a metal well reported to be difficult to be removed in bioreactors and other engineered wetland systems, was found to be much more labile (bound to the exchangeable and the carbonate fractions) and therefore potentially more available. XRD and SEM-EDS analyses also suggested that sulfide precipitation was taking place. Similarly, the adsorption study supported the SEP results and showed that the organic materials had a high potential to scavenge Mn through adsorption.

From a methodological point of view, the SEP used in combination with the geochemical model was very informative. And although frequent issues associated with SEP are difficult to

assess (e.g. limited selectivity of the reagents, possibility of re-adsorption and re-distribution of the extracted metals, insufficiency of reagent if the metal concentrations are too high), the precision of the methodology was found to be satisfactory and sufficiently reproducible for its purpose. On the other hand, the XRD and the SEM-EDS analyses proved to be less reliable and not very well suited to the nature of the samples. For example, the high heterogeneity of the spent substrates made it hard to identify metal precipitates. Furthermore, the sample preparation (i.e. overnight air drying) undoubtedly affected the speciation of the metals and the nature of the precipitates (e.g. a large amount of sulfide minerals may have been degraded during the drying process).

As briefly discussed in Chapters 3 and 4, issues associated with the ingress of oxygen through the pore-water port during sampling events likely led to less reduced microenvironments within the SRBR, and certainly promoted the precipitation of (oxy)hydroxides instead of sulfides (especially in short HRT systems). This hypothesis was supported by a visual examination of the empty reactors post-treatment (Figure 7-2 in Appendix B) clearly showing the presence of iron ochres close to the pore-water sampling ports inside the reactors.

Overall, systems operating at a longer HRT seemed to have promoted the precipitation of Fe, Ni and Cu sulfides as well as Mn and Zn carbonates. In a similar way, the SRBR using mussel shells instead of limestone seemed to have promoted the precipitation of metal sulfides (Fe, Ni, Cu, Zn) as well as Mn carbonates.

CHAPTER 6

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The main objectives of this research were to evaluate the treatment efficiencies of four different upward-flow SRBR systems treating MIW as a function of: (1) the alkalinity amendment used in the reactive substrate mixture (mussel shell or limestone); (2) the HRT (short HRT of ~ 3 days or long HRT of ~ 10 days).

This thesis comprised three results chapters presenting the physico-chemical and hydraulic characteristics of the substrate materials and their mixtures pre-treatment (Chapter 3), the MIW treatability tests, in particular the alkalinity generation, the sulfate, and the metal removal efficiencies (Chapter 4), and investigating the metal removal mechanisms through geochemical and mineralogical analyses of the spent substrate mixtures (Chapter 5).

6.1 Main findings

The hydraulic parameters (i.e. porosity and saturated hydraulic conductivity) indicated that approximately half of the substrate volume was occupied by void and therefore the risk of clogging and/or short-circuiting was estimated to be low. The chemical analyses showed that the metal contents were up to 7 times higher in the limestone compared to the mussel shells. This finding did not result in major dissimilarities in terms of SRBR's metal removal efficiencies (Chapter 4), but noticeable differences were observed during the autopsies of the spent substrates (Chapter 5). The pre-treatment mineralogical analyses indicated that mussel

shells were composed of a mixture of aragonite and calcite, while the limestone was made of pure calcite. This finding had strong implications in terms of alkalinity generation, as the mussel shells yield considerably more alkalinity than the limestone at both HRT.

The treatability tests showed that initially each of the four SRBR systems successfully treated the influent MIW, while in the long run, the system operating at a shorter HRT and containing limestone showed clear signs of malfunctioning (lower alkalinity generation, and lower sulfate and metal removal efficiencies). Overall, SRBR operating at a longer HRT were believed to be truly reduced system operating at steady-state equilibrium and containing a more active sulfate-reducing bacterial community, while the shorter HRT systems were constantly shifted from equilibrium due to the continuous addition of MIW. In addition, reactors containing mussel shells in the reactive mixture showed better treatment performances in terms of effluent pH, alkalinity generation, and metal removal. The sulfate reduction was positively affected by the HRT, but not by the alkalinity amendments.

The post-treatment autopsies showed that the SRBR retained large quantities of metals within the substrate mixtures, and that most metals were retained in a primary non-bioavailable form. The metal removal mechanisms are thought to have included precipitation of sulfides, (oxy)hydroxides, carbonates as well as adsorption. Mn was generally retained in a more labile form compared to the other metal of concerns, through both adsorption onto organic matter and precipitation of carbonate mineral.

Overall, this study demonstrated that:

(1) SRBR offers an attractive solution to MIW treatment, especially in the frequent cases of remotely located and/or abandoned mines. Although, these bioreactor systems are not always effective (for example Mn removal is still not fully understood and not always efficient, and hydraulic failures due to short-circuiting and clogging are still frequently reported), nor truly sustainable (regular replacement of the substrates and proper disposal of the residues must be done), they are one of the most promising passive technique available and the closest option to a self-regenerating treatment system (as the MIW generation is itself self-perpetuating, a self-regenerating treatment system is the ultimate goal);

(2) The use of complex organic substrates using waste materials (instead of highly refined organic substances such as ethanol or lactate) increases the sustainability of the system, but simultaneously its complexity;

(3) The use of waste mussel shell offers an attractive and sustainable alternative to limestone for MIW passive treatment in New Zealand and in other countries with access to similar seafood waste products. In addition, the results from Chapters 4 and 5 suggested that the use of mussel shell resulted in better treatment compared to the use of limestone in terms of alkalinity generation, metal removal and stability of the metal residues. These differences were mainly attributed to the unique mineralogy of the shells (high aragonite content);

(4) Even though an increase in HRT resulted in a better contaminant removal, an HRT_n of approximately 3 days was sufficient to remove about 80% of all metals (except Mn). Therefore, the difficult choice of an optimal HRT must balance the need to meet a specific effluent quality while keeping the treatment time reasonably short, and an intermediate retention time might have been optimal.

6.2 Limitations of the present study and future work recommendations

Aerobic microenvironments, even if only temporary, are frequent in bioreactors of this type. Nevertheless, it seems possible that the introduction of oxygen through the pore-water ports led to less reduced environments within the SRBR and could have promoted the precipitation of (oxy)hydroxides instead of sulfides.

Although the compost did inoculate the bioreactors with SRB and other fermenters necessary for the proper functioning of the systems, it was hypothesized that these microorganisms were not well adapted to the harsh conditions encountered in the reactors. This could have been addressed by using other types of inoculums such as riverbed or wetland sediments impacted by MIW, or spent substrates from well-functioning SRBR. Completely absent from the present study, microbial analyses should be conducted in the future to test if the use of mussel shells would result in important differences in terms of microbial diversity, total population and biological activity. In addition, ammonia - nitrogen (NH_3-N) should be monitored to better track the overall bacterial population activity.

Future research should also comprise more detailed investigations of the organic substrate materials and decomposition rates. This should include pre-treatment analyses of the materials (e.g. EAS, cellulose and lignin contents), and measurements of simple organic substances (e.g. EAS, or fatty volatile acids) leaching from the reactors. This would help evaluating different waste material characteristics and their effects on the system longevity. Place et al. (2005; 2006) established a sequential extraction procedure to evaluate the organic materials (it looked at the relative amount of EAS, cellulose and hemicellulose, and lignin in organic materials).

By conducting such experiments pre- and post-treatment, and in multiple locations within a treatment system, the substrate degradability could be studied at both temporal and spatial scales. In addition, a better understanding of factors affecting the complex sulfur transformations (sulfate reduction, sulfide generation and metal sulfide precipitation) is also needed. Quantifying the H_2S gas produced in SRBR could help establishing a complete mass balance, and isotopic studies could improve our current understanding of the numerous sulfur transformations occurring in SRBR (biotic versus abiotic processes).

Hydraulic problems related to short-circuiting and preferential flow path development were not studied in the present study, but are likely to have taken place, at least to a certain extent. This potentially resulted in real HRT changing over time and potentially shorter than the computed nominal HRT_n presented here. Tracer studies and hydrodynamic models could have helped evaluate such issues, as well as better estimating the initial HRT. Conducting such evaluations before and during treatment and after decommissioning the reactors would help gain knowledge on the physical transformations occurring in SRBR. It is recommended to undertake these tests on pilot or full-scale systems rather than on lab-scale systems as scaling up the treatment unit will undoubtedly have important repercussions on the hydraulic behavior. In addition, numerous design parameters such as flow-direction and regime (downward, upward, horizontal flow), size and shape of the systems, and operational temperature should be investigated as they will certainly have an influence on the hydraulic behaviors and the treatment performances of such systems.

Researching the above-mentioned questions, especially the hydraulic behavior and the organic substrate materials decomposition rates, would help addressing the complex implications related to scaling-up these systems to pilot and full-scale systems. In the long term, practical guidelines recommending size and operational design (i.e. hydraulic flow and regime), substrate composition (i.e. relative amounts of EAS vs. recalcitrant materials), and optimal HRT should be developed. Unfortunately, due to the complex interactions responsible for the treatment in SRBR, much work still needs to be undertaken before researchers can establish such guidelines. In addition, these guidelines would have to be fitted to local and regional conditions and would ultimately greatly vary depending on the MIW chemistry, the substrate composition (i.e. the decomposition rates), and the microbial activity.

The fact that Mn was more labile and potentially retained in more bioavailable forms raised questions regarding the appropriate disposal of the spent substrates (an often neglected aspect of such studies). Should the spent substrates be considered a hazardous waste? Can they be disposed of in conventional landfills, or do they require special handling and disposal practices before being buried? Such questions should be addressed through column leaching studies (studies which could conjointly examine the possibility of metal recovery through chemical or bio-leaching). A brief comparison between the total amount of metals retained within the spent substrates with the Landfill Waste Acceptance Criteria published by the New Zealand Ministry of Environment (MfE 2004) showed that the present spent substrates contained metal concentrations that were too high to be disposed of in conventional Class B landfill and also

potentially too high to be disposed of in conventional Class A landfills[1]. Where the contaminant concentrations exceed the screening values determined in the Landfill Waste Acceptance Criteria document, a Toxicity Characteristic Leaching Procedure (TCLP) test, as defined by the US-EPA, can be performed. This test will show if the contaminants are contained in an inert and satisfactorily immobilized form within the waste matrix. Therefore if a waste exceeds the solid waste threshold value, but is still under the leachate concentration values, it can be disposed of in Class A landfills. If it exceeds the leachate concentration values, then further treatment is required. Appendix A-7 shows the maximum metal concentrations allowed for the solid wastes and for the leachates in each type of landfill. In the present case, the spent substrates should be submitted to a TCLP test in order to evaluate if the leachate concentrations would exceed the values presented in Appendix A-7.

Finally, MIW treatment could in some cases be approached as an emerging resource. For examples, iron pigments and selective metals recovery have already been widely studied. Energy generation through the use of micro-hydro generators located at the effluent parts of a system could for example provide electricity to run continuously-logged analytical instruments in remote locations or maybe even to warm up a SRBR in particularly cold environments. This could contribute to the overall sustainability of such passive systems.

[1] Conventional Class A landfills have engineered systems able to collect leachate and gas, while Class B landfills have limited or no engineered system and may be located in areas posing risks for the environment (e.g. over permeable soil or gravel, in floodplains).

LIST OF APPENDICES

7.1 Appendix A: Raw and additional data

Appendix A-1: Water quality raw data including: in-situ parameters (pH, ORP, conductivity, temperature, dissolved oxygen), alkalinity and acidity, nutrients, carbon, sulfate, sulfide, and metals (influent, effluent and pore-water, total and dissolved concentrations).

Appendix A-2: Summary of two-way ANOVA statistical analyses.

Appendix A-3: SEP raw data.

Appendix A-4: Gas analyses.

Appendix A-5: Initial and final pH measured during the adsorption edge study.

Appendix A-6: PHREEQC input parameters.

Appendix A-7: PHREEQC geochemical modeling SI output values.

Appendix A-8: Waste acceptance screening criteria for Class A and Class B landfills and concentrations in leachate following the TCLP (MfE 2004)**.**

Appendix A-1: Water quality raw data for week 1 to 41. “Inf.” = influent, “Eff.” = effluent, “PW” = pore-water, “-“ = not measured

pH (-)

Week	Date (dd/mm/yy)	MIW batch	Inf.	Mussel shell Short HRT				Mussel shell Long HRT				Limestone Short HRT				Limestone Long HRT			
				SRBR 1		SRBR 2		SRBR 3		SRBR 4		SRBR 5		SRBR 6		SRBR 7		SRBR 8	
				Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW
1	18/03/13	1	3.34	6.80	6.78	6.76	6.81	6.52	6.49	6.51	6.52	6.32	6.28	6.52	6.48	6.29	6.31	6.43	6.45
2	25/03/13	1	3.67	6.75	6.67	6.57	6.54	6.48	6.45	6.57	6.61	6.44	6.36	6.25	6.33	6.32	6.29	6.25	6.32
3	2/04/13	1	4.96	6.80	6.74	7.03	6.98	6.67	6.63	7.21	6.97	7.03	6.87	6.87	6.76	6.47	6.44	6.77	6.58
4	11/04/13	1	4.96	7.21	6.92	7.05	7.01	7.06	6.88	7.11	7.06	6.77	6.71	6.73	6.67	6.62	6.57	6.54	6.52
6	22/04/13	2	3.99	6.77	6.71	6.71	6.35	6.84	6.59	6.73	6.61	6.66	6.79	6.59	6.50	6.78	6.49	6.51	6.51
7	29/04/13	2	2.98	6.56	6.73	6.77	6.24	6.78	6.67	6.84	6.63	6.68	6.69	6.47	6.37	6.81	6.44	7.28	6.45
8	6/5/13	2	2.95	6.53	6.34	6.72	6.46	6.68	6.64	6.60	6.67	6.52	6.66	6.41	6.43	6.45	6.41	6.46	6.39
9	13/5/13	2	2.89	6.45	6.03	6.64	6.37	6.66	6.70	6.67	6.77	6.46	6.46	6.39	6.34	6.54	6.65	6.55	6.46
10	21/5/13	2	2.91	6.47	6.47	6.64	6.15	6.74	6.73	6.68	6.72	6.27	6.60	6.20	6.33	6.51	6.47	6.50	6.48
11	27/5/13	2	2.89	6.56	6.26	6.72	6.22	6.83	6.78	6.80	6.73	6.44	6.51	6.49	6.40	6.62	6.49	6.51	6.49
12	3/6/13	2	2.87	6.38	6.28	6.60	6.34	6.76	6.77	6.70	6.78	6.44	6.47	6.46	6.53	6.48	6.47	6.49	6.48
13	10/6/13	2	2.92	6.63	6.47	6.77	6.28	6.84	6.81	6.83	6.77	6.57	6.57	6.46	6.53	6.61	6.53	6.74	6.53
14	17/6/13	3	2.96	6.26	5.55	6.62	6.00	6.91	6.73	6.98	6.71	6.39	5.98	6.33	6.38	6.48	6.44	7.00	6.39
15	24/6/13	3	2.93	6.19	5.28	6.66	6.06	6.85	6.79	6.82	6.74	6.16	6.36	6.29	6.34	6.44	6.36	6.53	6.48
16	1/7/13	3	2.89	6.05	5.91	6.63	5.85	6.65	6.67	6.81	6.64	5.71	6.15	5.69	6.21	6.21	6.23	6.24	6.35
17	8/7/13	3	2.90	5.92	6.33	6.64	6.03	6.67	6.72	6.62	6.72	5.89	6.35	6.18	6.36	6.24	6.27	6.29	6.44
18	15/7/13	3	2.89	6.16	6.13	6.56	5.53	6.66	6.62	6.57	6.60	6.18	6.03	6.26	6.28	6.13	6.11	6.07	6.33
19	22/7/13	3	2.87	6.17	5.74	6.57	6.14	6.78	6.68	7.04	6.66	6.18	6.31	6.32	6.43	6.38	6.34	6.31	6.39
20	29/7/13	3	2.79	6.21	6.42	6.55	6.30	6.69	6.71	7.22	6.74	6.26	6.36	5.73	6.40	6.41	6.42	6.07	6.39
22	21/10/13	4	2.87	-	-	6.57	6.12	6.51	6.79	-	-	6.14	6.22	-	-	6.54	6.38	-	-
24	4/11/13	4	2.91	-	-	6.64	6.05	6.61	6.76	-	-	6.01	6.37	-	-	6.46	6.49	-	-
27	25/11/13	4	2.76	-	-	6.51	6.04	6.63	6.93	-	-	5.56	6.09	-	-	6.26	6.45	-	-
29	9/12/13	4	2.75	-	-	6.37	6.21	6.61	6.72	-	-	4.42	5.86	-	-	5.78	6.65	-	-
31	20/12/13	5	2.90	-	-	6.38	5.95	6.66	6.83	-	-	5.71	5.34	-	-	6.36	6.57	-	-

33	7/1/14	5	2.88	-	-	6.26	6.06	6.73	6.75	-	-	4.24	5.16	-	-	5.98	6.39	-	-
35	21/1/14	5	2.81	-	-	6.17	6.17	6.69	6.74	-	-	4.99	5.63	-	-	5.95	6.34	-	-
37	2/2/14	5	2.83	-	-	6.29	5.97	6.72	6.67	-	-	3.80	4.80	-	-	5.77	6.54	-	-
39	17/2/14	5	2.82	-	-	6.02	6.25	6.83	6.79	-	-	3.86	4.61	-	-	5.70	6.42	-	-
41	3/3/14	5	2.86	-	-	5.94	6.25	6.73	6.88	-	-	4.10	4.69	-	-	5.93	6.54	-	-

ORP (mV)

Week	MIW batch	Inf.	Mussel shell Short HRT				Mussel shell Long HRT				Limestone Short HRT				Limestone Long HRT			
			SRBR 1		SRBR 2		SRBR 3		SRBR 4		SRBR 5		SRBR 6		SRBR 7		SRBR 8	
			Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW
1	1	294	-322	-301	-208	-224	-309	-326	-310	-289	-207	-227	-246	-255	-247	-278	-265	-271
2	1	239	-198	-254	-229	-261	-238	-301	-246	-265	-206	-178	-221	-238	-155	-224	-190	-231
3	1	187	-208	-283	-196	-209	-166	-287	-137	-237	-186	-188	-124	-245	-179	-215	-185	-208
4	1	129	-27	-152	-156	-198	-129	-254	-130	-205	-96	-157	-151	-226	-149	-222	-157	-212
6	2	203	-209	-167	-24	-142	-24	-234	-145	-224	-174	-170	-218	-201	-92	-226	-124	-222
7	2	431	-76	-131	-61	-124	-84	-213	-130	-219	-117	-64	-36	-132	-54	-214	-100	-218
8	2	432	-175	-115	-120	-169	-174	-196	-194	-197	-152	-155	-109	-161	-89	-187	-108	-200
9	2	485	112	100	28	14	42	-185	-145	-207	-120	-112	-24	-52	-117	-193	17	-205
10	2	461	6	-110	-46	-104	-81	-181	-173	-181	-127	-116	-2	-62	-65	-151	-132	-195
11	2	458	-82	-79	0	-103	-8	-212	-162	-213	-129	-116	-81	-102	-31	-201	-157	-198
12	2	434	-37	-60	10	-59	-24	-214	-123	-204	-26	-130	-60	-146	-58	-191	-100	-192
13	2	456	26	68	-96	-128	-56	-211	-148	-223	-168	-121	29	-147	-41	-207	-107	-220
14	3	491	57	165	86	134	-166	-120	-40	-210	-72	-60	-34	-132	-147	-175	-18	-189
15	3	469	72	106	110	86	-194	-197	-96	-166	-24	-69	-11	-104	-16	-153	-24	-166
16	3	480	86	102	56	66	90	-177	-24	-110	118	3	165	-90	-32	-118	29	-167
17	3	486	87	-100	76	-24	57	-147	43	-72	57	-87	63	-144	63	-143	57	-187
18	3	465	38	-54	38	-105	63	-218	54	-194	-95	-93	-116	-176	-125	-200	2	-222
19	3	457	-51	-113	-65	-135	-9	-205	10	-204	49	-129	54	-147	33	-168	34	-191
20	3	459	95	-146	65	-122	76	-220	81	-88	115	-143	141	-148	121	-207	83	-214
22	4	509	-	-	29	199	-267	-220	-	-	195	177	-	-	132	-243	-	-

24	4	473	-	-	-43	-96	45	-257	-	-	4	-148	-	-	7	-244	-	-
27	4	442	-	-	80	-99	96	-323	-	-	41	-28	-	-	-19	-116	-	-
29	4	489	-	-	90	101	-221	-322	-	-	202	-142	-	-	-18	-107	-	-
31	5	468	-	-	137	192	-278	-283	-	-	149	142	-	-	-153	-195	-	-
33	5	468	-	-	86	-5	-284	-319	-	-	124	-129	-	-	-116	-248	-	-
35	5	435	-	-	69	-144	34	-303	-	-	132	-157	-	-	-16	-253	-	-
37	5	473	-	-	40	-70	-241	-322	-	-	365	-132	-	-	-165	-276	-	-
39	5	439	-	-	-183	-126	-121	-273	-	-	236	-53	-	-	-54	-253	-	-
41	5	479	-	-	-120	-16	-180	-304	-	-	95	8	-	-	-68	-279	-	-

Conductivity (mS)

Week	MIW batch	Inf.	Mussel shell Short HRT				Mussel shell Long HRT				Limestone Short HRT				Limestone Long HRT			
			SRBR 1		SRBR 2		SRBR 3		SRBR 4		SRBR 5		SRBR 6		SRBR 7		SRBR 8	
			Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW
1	1	1.25	1.62	-	1.57	-	1.56	-	1.59	-	1.36	-	1.35	-	1.39	-	1.53	-
2	1	1.23	1.52	-	1.64	-	1.65	-	1.67	-	1.44	-	1.47	-	1.31	-	1.39	-
3	1	1.26	1.40	-	1.40	-	1.37	-	1.35	-	1.14	-	1.15	-	1.17	-	1.15	-
4	1	1.32	1.24	-	1.26	-	1.39	-	1.40	-	1.16	-	1.16	-	1.20	-		-
6	2	1.94	1.47	1.93	1.59	2.03	1.37	1.61	1.36	1.67	1.48	1.87	1.55	1.88	1.12	1.24		1.20
7	2	2.11	2.06	1.83	2.11	2.08	1.93	2.15	1.92	2.13	1.99	1.97	1.98	1.99	1.16	1.88	1.13	1.88
8	2	2.16	2.00	2.01	2.07	2.05	2.09	2.11	2.06	2.10	1.96	1.98	1.94	1.98	1.96	2.03	1.94	2.00
9	2	2.13	1.92	1.86	1.97	1.92	2.02	2.03	2.00	2.02	1.87	1.89	1.88	1.89		1.96	1.90	1.94
10	2	2.61	2.34	2.41	2.39	2.40	2.46	2.47	2.43	2.44	2.27	2.29	2.26	2.31	2.32	2.37	2.29	2.34
11	2	2.60	2.28	2.29	2.34	2.29	2.38	2.40	2.35	2.37	2.23	2.23	2.23	2.25	2.25	2.29	2.24	2.29
12	2	2.56	2.27	2.26	2.30	2.29	2.35	2.37	2.32	2.35	2.21	2.24	2.24	2.25	2.21	2.26	2.20	2.24
13	2	2.61	2.30	2.25	2.35	2.28	2.34	2.40	2.33	2.36	2.24	2.22	2.25	2.25	2.23	2.28	2.21	2.26
14	3	2.81	2.52	2.53	2.58	2.57	2.44	2.64	2.44	2.61	2.45	2.54	2.48	2.55	2.39	2.55	2.36	2.56
15	3	2.76	2.44	2.41	2.58	2.50	2.51	2.60	2.52	2.56	2.43	2.46	2.43	2.52	2.42	2.47	2.42	2.46
16	3	2.82	2.48	2.50	2.63	2.52	2.65	2.69	2.58	2.62	2.43	2.48	2.44	2.54	2.45	2.50	2.44	2.50
17	3	2.48	2.17	2.26	2.30	2.26	2.28	2.37	2.25	2.33	2.13	2.18	2.18	2.22	2.12	2.18	2.14	2.20

18	3	2.37	2.09	2.09	2.18	2.08	2.21	2.22	2.13	2.20	2.04	2.04	2.06	2.09	2.01	2.05	2.03	2.06
19	3	2.46	2.17	2.14	2.31	2.21	2.29	2.33	2.26	2.32	2.17	2.16	2.17	2.22	2.10	2.15	2.09	2.15
20	3	2.46	2.13	2.30	2.24	2.29	2.24	2.30	2.23	2.31	2.06	2.17	2.05	2.16	2.07	2.14	2.03	2.14
22	4	2.26	-	-	2.06	1.98	2.06	2.11	-	-	1.93	1.97	-	-	1.99	2.01	-	-
24	4	2.32	-	-	2.06	2.03	2.08	2.11	-	-	1.94	2.00	-	-	1.97	2.01	-	-
27	4	2.45	-	-	2.11	2.08	2.13	2.18	-	-	1.97	2.03	-	-	1.96	2.04	-	-
29	4	2.56	-	-	2.10	2.10	2.14	2.21	-	-	1.96	2.07	-	-	1.95	2.06	-	-
31	5	2.42	-	-	2.07	2.07	2.10	2.17	-	-	1.98	2.06	-	-	1.99	2.08	-	-
33	5	2.62	-	-	2.24	2.21	2.39	2.40	-	-	2.15	2.23	-	-	2.14	2.24	-	-
35	5	2.60	-	-	2.17	2.19	2.29	2.34	-	-	2.09	2.17	-	-	2.08	2.16	-	-
37	5	2.61	-	-	2.17	2.14	2.32	2.35	-	-	2.13	2.15	-	-	2.06	2.17	-	-
39	5	2.69	-	-	2.23	2.21	2.39	2.42	-	-	2.19	2.29	-	-	2.11	2.20	-	-
41	5	2.58	-	-	2.10	2.09	2.22	2.30	-	-	2.07	2.16	-	-	2.01	2.10	-	-

Temperature (°C)

Week	MIW batch	Inf.	Mussel shell Short HRT				Mussel shell Long HRT				Limestone Short HRT				Limestone Long HRT			
			SRBR 1		SRBR 2		SRBR 3		SRBR 4		SRBR 5		SRBR 6		SRBR 7		SRBR 8	
			Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW
1	1	18.8	18.8	-	18.8	-	18.8	-	18.8	-	18.8	-	18.8	-	18.8	-	18.9	-
2	1	18.7	18.5	-	18.5	-	18.5	-	18.5	-	18.5	-	18.3	-	18.4	-	18.3	-
3	1	17.2	18.7	-	17.5	-	18.4	-	18.3	-	18.0	-	18.2	-	18.2	-	18.3	-
4	1	17.5	15.6	-	15.7	-	15.6	-	15.7	-	15.8	-	15.8	-	15.8	-	15.7	-
6	2	16.9	15.4	15.8	16.6	16.5	15.4	15.7	15.4	15.7	15.2	15.7	15.4	15.8	15.4	15.8	15.5	15.7
7	2	16.3	17.2	17.2	17.1	17.2	16.9	17.1	17.0	17.2	17.2	17.2	16.9	17.3	17.0	17.3	17.1	17.2
8	2	16.3	16.3	16.5	16.1	16.3	15.9	16.3	16.0	16.3	16.2	16.3	16.0	16.4	16.0	16.5	16.0	16.4
9	2	15.0	14.5	14.7	14.5	14.7	14.7	14.8	14.6	15.0	14.5	14.8	14.7	14.8	14.6	14.8	14.7	14.9
10	2	15.6	15.6	15.7	15.5	15.6	15.5	15.7	15.6	15.7	15.5	15.7	15.4	15.7	15.7	15.7	15.6	15.7
11	2	15.3	14.2	14.6	14.2	14.6	14.3	14.6	14.3	14.7	14.4	14.6	14.3	14.4	14.2	14.5	14.4	14.7
12	2	14.5	14.0	14.1	14.1	14.1	13.9	14.0	13.9	13.9	13.8	14.0	13.9	14.0	13.8	13.9	14.0	14.1
13	2	14.8	14.0	14.1	14.0	14.1	13.9	14.2	14.1	14.3	13.9	14.0	13.9	14.2	14.0	14.2	14.0	14.3

14	3	15.0	14.3	14.5	14.4	14.6	14.4	14.7	14.4	14.7	14.3	14.6	14.3	14.6	14.3	14.6	14.6	14.6
15	3	13.1	11.9	12.3	12.0	12.2	12.1	12.6	12.2	12.4	12.0	12.4	12.1	12.5	12.1	12.3	12.3	12.3
16	3	13.7	12.2	12.7	12.3	12.6	12.5	12.8	12.9	12.9	12.5	12.8	12.4	12.8	12.6	12.6	15.2	12.8
17	3	16.3	15.1	15.5	15.0	15.3	14.9	15.5	15.2	15.4	15.1	15.4	15.1	15.4	15.1	15.4	15.2	15.5
18	3	14.6	13.2	13.5	13.3	13.6	13.5	13.7	13.3	13.6	13.2	13.6	13.6	13.6	13.5	13.5	13.5	13.6
19	3	16.0	14.9	15.0	14.8	15.1	15.0	15.2	14.9	15.2	15.0	15.1	15.0	15.1	15.0	15.2	15.0	15.2
20	3	15.5	14.3	14.7	13.8	14.3	14.1	14.8	14.3	14.8	13.8	14.6	13.6	14.7	13.9	14.8	14.0	14.6
22	4	17.5	-	-	17.0	17.3	16.9	17.7	-	-	16.9	17.3	-	-	17.5	17.8	-	-
24	4	17.9	-	-	16.6	17.0	17.4	17.5	-	-	16.7	17.1	-	-	17.0	17.4	-	-
27	4	18.5	-	-	18.4	18.7	18.3	18.7	-	-	18.2	18.7	-	-	18.3	18.7	-	-
29	4	20.4	-	-	19.3	20.0	19.1	19.9	-	-	18.8	19.7	-	-	19.3	19.0	-	-
31	5	18.2	-	-	17.5	17.7	17.7	17.9	-	-	17.6	17.9	-	-	18.0	18.2	-	-
33	5	20.2	-	-	19.6	19.8	19.3	19.7	-	-	19.5	19.9	-	-	19.4	19.9	-	-
35	5	19.0	-	-	18.2	18.8	18.0	18.7	-	-	18.2	18.7	-	-	18.3	18.8	-	-
37	5	19.0	-	-	18.3	18.7	18.5	18.8	-	-	18.4	18.6	-	-	18.5	18.8	-	-
39	5	20.0	-	-	19.4	19.7	19.7	19.9	-	-	19.6	19.8	-	-	19.6	20.0	-	-
41	5	17.3	-	-	17.0	17.1	16.9	17.1	-	-	16.9	17.1	-	-	17.0	17.2	-	-

Dissolved oxygen (%)

Week	**MIW batch**	**Inf.**	**Mussel shell Short HRT**				**Mussel shell Long HRT**				**Limestone Short HRT**				**Limestone Long HRT**			
			SRBR 1		**SRBR 2**		**SRBR 3**		**SRBR 4**		**SRBR 5**		**SRBR 6**		**SRBR 7**		**SRBR 8**	
			Eff.	**PW**	**Eff.**	**PW**	**Eff.**	**PW**	**Eff.**	**PW**	**Eff.**	**PW**	**Eff.**	**PW**	**Eff.**	**PW**	**Eff.**	**PW**
21	4	98.2	-	-	35.7	11.2	20.2	12.3	-	-	74.0	11.3	-	-	57.8	13.2	-	-
22	4	97.0	-	-	51.6	11.9	19.4	11.4	-	-	98.0	12.8	-	-	60.1	11.5	-	-
24	4	99.8	-	-	30.2	23.8	53.2	8.9	-	-	57.8	13.8	-	-	60.8	14.6	-	-
27	4	89.7	-	-	32.5	23.0	23.6	2.2	-	-	52.7	14.5	-	-	45.6	12.5	-	-
29	4	91.2	-	-	56.1	6.9	19.6	6.1	-	-	58.7	17.2	-	-	53.5	21.5	-	-
31	5	95.6	-	-	53.1	18.5	29.1	5.1	-	-	60.1	9.1	-	-	45.9	18.7	-	-
33	5	98.7	-	-	40.4	15.0	28.2	3.0	-	-	68.6	7.8	-	-	48.2	8.1	-	-
35	5	90.7	-	-	39.7	17.3	17.8	3.0	-	-	59.2	12.5	-	-	62.8	27.8	-	-

37	5	99.2	-	-	56.6	32.1	22.7	4.4	-	-	76.8	30.6	-	-	72.6	26.3	-	-
39	5	97.9	-	-	59.1	29.9	28.6	6.4	-	-	81.9	27.5	-	-	74.7	25.3	-	-
41	5	96.5	-	-	59.6	27.2	46.8	4.3	-	-	83.7	19.8	-	-	82.7	9.1	-	-

Acidity and Alkalinity (mg/L $CaCO_3$ eq.)

				Mussel shell Short HRT		Mussel shell Long HRT		Limestone Short HRT		Limestone Long HRT	
Week	**MIW batch**	**Mineral acidity**	**Total acidity**	**SRBR 1**	**SRBR 2**	**SRBR 3**	**SRBR 4**	**SRBR 5**	**SRBR 6**	**SRBR 7**	**SRBR 8**
		Inf.	**Inf.**	**Eff.**	**Eff.**	**Eff.**	**Eff.**	**Eff.**	**Eff.**	**Eff.**	**Eff.**
1	1	-	-	528.8	316.3	552.5	608.8	322.5	316.3	353.8	531.8
2	1	-	-	522.5	665.0	712.5	737.5	515.0	525.0	275.0	380.0
3	1	-	-	380.0	343.8	493.8	556.3	-	245.0	402.5	437.5
4	1	-	-	251.3	302.5	478.8	490.0	173.5	185.0	313.8	302.5
6	2	190.0	293.0	573.8	685.0	702.5	670.0	327.5	312.5	540.0	-
7	2	55.5	182.5	190.0	297.5	617.5	607.5	147.5	135.0	667.5	635.0
8	2	115.5	260.5	210.0	260.0	420.0	437.5	140.0	132.5	317.5	337.5
9	2	116.5	262.0	187.5	220.0	395.0	407.5	120.0	120.0	-	245.0
10	2	111.0	262.0	167.5	187.5	352.5	355.0	90.0	77.5	285.0	270.0
11	2	112.5	274.5	170.0	230.0	352.5	357.5	92.5	100.0	245.0	227.5
12	2	113.5	257.0	190.0	187.5	360.0	362.5	130.0	135.0	207.5	197.5
13	2	106.5	257.0	205.0	230.0	362.5	342.5	147.5	147.5	302.5	307.5
14	3	226.0	469.0	147.5	230.0	395.0	390.0	112.5	110.0	282.5	287.5
15	3	221.0	443.5	117.5	232.5	372.5	325.0	82.5	90.0	180.0	120.0
16	3	216.5	440.0	117.5	240.0	310.0	110.0	27.5	40.0	132.5	140.0
17	3	221.0	467.0	80.0	232.5	237.5	182.5	40.0	97.5	117.5	135.0
18	3	214.5	480.0	127.5	230.0	315.0	255.0	67.5	42.5	122.5	140.0
19	3	216.5	453.0	147.5	287.5	350.0	340.0	77.5	80.0	177.5	122.5
20	3	223.0	475.5	132.5	277.5	365.0	356.5	72.5	47.5	217.5	110.0
22	4	184.5	354.5	-	232.5	360.0	-	97.5	-	277.5	-
24	4	186.0	352.5	-	182.5	272.5	-	55.0	-	167.5	-

27	4	181.0	364.0	-	152.5	242.5	-	22.5	-	97.5	-
29	4	187.5	371.5	-	112.5	220.0	-	2.5	-	35.0	-
31	5	162.5	339.5	-	140.0	270.0	-	32.5	-	147.5	-
33	5	160.5	348.0	-	95.0	227.5	-	0.0	-	55.0	-
35	5	164.0	338.5	-	120.0	270.0	-	5.0	-	55.0	-
37	5	161.0	333.0	-	105.0	260.0	-	0.0	-	32.5	-
39	5	160.5	340.0	-	80.0	262.5	-	0.0	-	30.0	-
41	5	161.0	335.5	-	57.5	212.5	-	7.5	-	37.5	-

Nitrates (mg/L)

Week	MIW batch	Inf.	Mussel shell Short HRT				Mussel shell Long HRT				Limestone Short HRT				Limestone Long HRT			
			SRBR 1		SRBR 2		SRBR 3		SRBR 4		SRBR 5		SRBR 6		SRBR 7		SRBR 8	
			Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW
22	4	7.5	-	-	1.7	1.5	1.5	1.9	-	-	0.5	1.2	-	-	0.6	1.0	-	-
24	4	7.3	-	-	1.8	1.6	0.7	1.8	-	-	0.7	1.3	-	-	0.3	0.7	-	-
27	4	6.5	-	-	1.5	1.7	0.9	1.7	-	-	1.3	1.5	-	-	1.8	1.3	-	-
29	4	6.4	-	-	1.1	1.6	1.5	1.8	-	-	0.7	3.1	-	-	1.4	3.2	-	-
31	5	5.2	-	-	1.2	1.8	3.9	3.5	-	-	1.6	1.4	-	-	1.5	1.2	-	-
33	5	6.8	-	-	1.5	2.3	1.6	2.4	-	-	1.1	4.1	-	-	0.9	2.0	-	-
35	5	5.4	-	-	1.6	1.7	1.2	3.1	-	-	1.2	1.9	-	-	1.0	3.0	-	-
37	5	6.7	-	-	2.6	2.0	1.6	7.1	-	-	1.3	5.6	-	-	1.3	4.8	-	-
39	5	6.6	-	-	2.2	1.6	1.2	5.5	-	-	0.8	1.8	-	-	1.2	5.9	-	-
41	5	5.8	-	-	2.8	3.1	1.7	2.5	-	-	0.9	1.5	-	-	1.2	1.8	-	-

Phosphates (mg/L)

Week	MIW batch	Inf.	Mussel shell Short HRT				Mussel shell Long HRT				Limestone Short HRT				Limestone Long HRT			
			SRBR 1		SRBR 2		SRBR 3		SRBR 4		SRBR 5		SRBR 6		SRBR 7		SRBR 8	
			Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW
22	4	0.2	-	-	2.5	43.5	7.0	17.5	-	-	1.0	11.0	-	-	1.0	38.5	-	-
24	4	0.2	-	-	3.0	131.5	2.0	31.5	-	-	2.0	195.0	-	-	1.5	25.5	-	-

27	4	0.4	-	-	2.5	13.5	6.5	141.0	-	-	3.5	21.0	-	-	3.5	48.5	-	-
29	4	0.4	-	-	2.5	108.5	7.0	102.0	-	-	1.0	70.0	-	-	2.0	74.0	-	-
31	5	1.0	-	-	6.0	48.0	7.0	102.0	-	-	0.0	62.0	-	-	2.5	72.0	-	-
33	5	2.0	-	-	4.0	42.0	6.0	94.0	-	-	1.0	132.0	-	-	3.5	60.0	-	-
35	5	0.5	-	-	6.0	34.0	4.5	86.0	-	-	1.0	60.0	-	-	1.0	18.0	-	-
37	5	1.0	-	-	11.0	32.0	5.5	38.0	-	-	1.0	16.0	-	-	1.5	18.0	-	-
39	5	1.0	-	-	11.0	18.0	5.5	86.0	-	-	1.0	22.0	-	-	1.5	24.0	-	-
41	5	1.0	-	-	7.5	18.0	5.5	102.0	-	-	1.0	16.0	-	-	1.5	36.0	-	-

COD (mg/L)

Week	MIW batch	Inf.	Mussel shell Short HRT				Mussel shell Long HRT				Limestone Short HRT				Limestone Long HRT			
			SRBR 1		SRBR 2		SRBR 3		SRBR 4		SRBR 5		SRBR 6		SRBR 7		SRBR 8	
			Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW
6	2	29.0	124.0	-	201.0	-	180.0	-	164.0	-	165.0	-	139.0	-	214.0	-		-
7	2	32.0	93.0	-	105.0	-	186.0	-	207.0	-	106.0	-	89.0	-	285.0	-	279.0	-
8	2	31.0	65.0	59.0	79.0	60.0	108.0	86.0	113.0	85.0	48.0	72.0	41.0	58.0	119.0	94.0	122.0	101.0
9	2	23.0	74.0	75.0	74.0	81.0	101.0	115.0	111.0	107.0	87.0	131.0	81.0	133.0	136.0	141.0	114.0	136.0
10	2	16.0	27.0	35.0	25.0	47.0	59.0	63.0	76.0	66.0	39.0	141.0	38.0	39.0	72.0	67.0	90.0	88.0
11	2	22.0	33.0	24.0	40.0	13.0	63.0	56.0	71.0	49.0	36.0	42.0	45.0	31.0	76.0	48.0	64.0	84.0
12	2	30.0	75.0	51.0	48.0	15.0	83.0	62.0	85.0	57.0	52.0	46.0	60.0	57.0	67.0	72.0	98.0	84.0
13	2	18.0	26.0	56.0	28.0	38.0	57.0	104.0	68.0	67.0	42.0	59.0	45.0	39.0	80.0	74.0	90.0	71.0
14	3	20.0	46.0	41.0	53.0	27.0	80.0	38.0	94.0	41.0	54.0	26.0	54.0	52.0	98.0	42.0	89.0	56.0
15	3	33.0	42.0	34.0	56.0	37.0	89.0	47.0	82.0	39.0	67.0	51.0	63.0	52.0	77.0	57.0	63.0	52.0
16	3	22.0	28.0	37.0	43.0	27.0	46.0	33.0	51.0	39.0	15.0	31.0	34.0	52.0	36.0	53.0	44.0	64.0
17	3	31.0	43.0	87.0	56.0	99.0	49.0	90.0	67.0	83.0	48.0	86.0	50.0	72.0	65.0	72.0	58.0	74.0
18	3	33.0	15.0	30.0	25.0	23.0	30.0	44.0	28.0	45.0	37.0	28.0	39.0	53.0	43.0	23.0	44.0	49.0
19	3	26.0	42.0	34.0	101.0	45.0	62.0	69.0	48.0	55.0	27.0	78.0	29.0	40.0	44.0	60.0	41.0	73.0
20	3	28.0	46.0	40.0	76.0	31.0	75.0	91.0	94.0	63.0	59.0	45.0	78.0	41.0	80.0	64.0	87.0	65.0

TOC (mg/L)

Week	MIW batch	Inf.	Mussel shell Short HRT				Mussel shell Long HRT				Limestone Short HRT				Limestone Long HRT			
			SRBR 1		SRBR 2		SRBR 3		SRBR 4		SRBR 5		SRBR 6		SRBR 7		SRBR 8	
			Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW
22	4	17.85	-	-	35.70	-	38.70	-	-	-	28.60	-	-	-	38.60	-	-	-
24	4	18.81	-	-	32.07	-	31.20	-	-	-	26.31	-	-	-	33.97	-	-	-
27	4	11.74	-	-	44.79	-	65.50	-	-	-	26.19	-	-	-	63.22	-	-	-
29	4	5.13	-	-	78.40	-	125.90	-	-	-	10.14	-	-	-	56.95	-	-	-
31	5	4.17	-	-	8.75	-	15.85	-	-	-	7.03	-	-	-	14.30	-	-	-
33	5	2.91	-	-	8.10	-	11.11	-	-	-	4.38	-	-	-	12.54	-	-	-
35	5	6.93	-	-	42.44	-	54.20	-	-	-	20.07	-	-	-	27.29	-	-	-
37	5	7.23	-	-	28.43	-	46.36	-	-	-	15.06	-	-	-	17.56	-	-	-
39	5	3.05	-	-	17.63	-	40.85	-	-	-	7.08	-	-	-	13.95	-	-	-
41	5	2.77	-	-	20.84	-	23.09	-	-	-	11.29	-	-	-	11.68	-	-	-

DOC (mg/L)

Week	MIW batch	Inf.	Mussel shell Short HRT				Mussel shell Long HRT				Limestone Short HRT				Limestone Long HRT			
			SRBR 1		SRBR 2		SRBR 3		SRBR 4		SRBR 5		SRBR 6		SRBR 7		SRBR 8	
			Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW
22	4	12.30	-	-	11.20	-	28.90	-	-	-	21.30	-	-	-	38.60	-	-	-
24	4	11.61	-	-	10.00	-	24.34	-	-	-	19.16	-	-	-	28.70	-	-	-
27	4	8.23	-	-	37.28	-	55.54	-	-	-	13.36	-	-	-	23.08	-	-	-
29	4	4.66	-	-	5.52	-	123.23	-	-	-	1.98	-	-	-	49.58	-	-	-
31	5	3.82	-	-	8.40	-	13.50	-	-	-	6.42	-	-	-	12.66	-	-	-
33	5	2.89	-	-	5.65	-	11.01	-	-	-	4.22	-	-	-	7.05	-	-	-
35	5	7.71	-	-	36.41	-	34.90	-	-	-	12.18	-	-	-	24.16	-	-	-
37	5	6.86	-	-	24.68	-	42.79	-	-	-	12.72	-	-	-	17.16	-	-	-
39	5	2.05	-	-	13.89	-	33.10	-	-	-	6.56	-	-	-	13.21	-	-	-
41	5	2.79	-	-	5.92	-	19.64	-	-	-	10.07	-	-	-	8.09	-	-	-

Sulfates (mg/L)

Week	MIW batch	Inf.	Mussel shell Short HRT				Mussel shell Long HRT				Limestone Short HRT				Limestone Long HRT			
			SRBR 1		SRBR 2		SRBR 3		SRBR 4		SRBR 5		SRBR 6		SRBR 7		SRBR 8	
			Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW
1	1	880	450.0	-	460.0	-	420.0	-	370.0	-	540.0	-	540.0	-	540.0	-	430.0	-
2	1	855	470.0	-	500.0	-	450.0	-	380.0	-	520.0	-	535.0	-	530.0	-	470.0	-
3	1	820	520.0	-	530.0	-	430.0	-	360.0	-	550.0	-	540.0	-	540.0	-	460.0	-
4	1	740	750.0	-	700.0	-	550.0	-	580.0	-	720.0	-	710.0	-	580.0	-	580.0	-
6	2	1800	600.0	1540.0	560.0	1500.0	200.0	1050.0	500.0	850.0	1050.0	1800.0	1000.0	1600.0	1200.0	500.0	1250.0	1350.0
7	2	1550	1400.0	1450.0	1450.0	1550.0	600.0	1300.0	550.0	1250.0	1300.0	1400.0	1400.0	1350.0	1110.0	1270.0	900.0	1380.0
8	2	1600	1350.0	1400.0	1300.0	1550.0	1150.0	1350.0	1150.0	1350.0	1400.0	1400.0	1400.0	1375.0	1225.0	1350.0	1250.0	1200.0
9	2	1550	1350.0	1425.0	1300.0	1400.0	1200.0	1350.0	1150.0	1350.0	1450.0	1350.0	1400.0	1400.0	1145.0	1400.0	1150.0	1150.0
10	2	1549	1466.0	1522.0	1499.0	1533.6	1340.8	1442.0	1330.0	1433.6	1470.8	1568.8	1491.1	1539.6	1298.0	1444.4	1293.6	1356.8
11	2	1528	1456.8	1529.2	1481.2	1542.4	1364.8	1428.0	1300.4	1468.4	1472.8	1546.8	1499.2	1515.6	1370.0	1389.2	1352.2	1382.0
12	2	1488	1399.6	1471.2	1425.2	1466.4	1282.4	1371.2	1230.0	1340.0	1421.2	1496.8	1434.4	1444.0	1309.2	1369.2	1307.6	1303.6
13	2	1514	1432.0	1579.2	1460.4	1506.4	1303.2	1408.8	1280.0	1434.0	1416.4	1517.2	1437.6	1475.2	1233.2	1344.0	1207.6	1287.2
14	3	1795	1633.6	1781.2	1572.4	1764.4	1278.4	1605.6	1272.8	1608.0	1605.2	1768.0	1628.0	1731.2	1351.2	1660.8	1286.0	1573.2
15	3	1777	1735.2	1898.8	1781.6	1838.8	1534.8	1741.2	1566.0	1731.2	1727.6	1818.8	1754.8	1802.8	1612.4	1750.4	1658.4	1626.0
16	3	1813	1751.2	1854.8	1757.2	1858.8	1675.2	1772.0	1676.8	1767.6	1768.8	1853.6	1730.4	1768.8	1664.4	1759.6	1657.2	1678.4
17	3	1822	1756.4	1819.6	1777.2	1837.2	1725.6	1768.0	1738.8	1789.6	1772.4	1808.0	1750.4	1727.6	1675.2	1737.6	1671.6	1629.6
18	3	1780	1746.4	1799.2	1740.0	1792.4	1679.4	1725.2	1722.6	1730.0	1746.8	1789.6	1754.0	1751.6	1636.4	1770.0	1656.0	1669.6
19	3	1994	1776.4	1904.0	1791.2	1877.2	1732.8	1770.8	1701.6	1757.2	1862.4	1861.6	1868.0	1846.4	1674.0	1771.6	1722.0	1709.2
20	3	1841	1750.8	1776.0	1709.2	1811.2	1625.2	1630.4	1606.4	1646.4	1758.4	1801.2	1758.8	1724.8	1570.8	1608.4	1581.6	1578.4
22	4	1471	-	-	1403.2	1562.0	1274.4	1244.8	-	-	1443.6	1521.6	-	-	1214.4	1343.6	-	-
24	4	1529	-	-	1452.4	1582.8	1348.4	1408.0	-	-	1468.4	1565.2	-	-	1365.5	1359.2	-	-
27	4	1523	-	-	1488.4	1564.4	1385.6	1438.4	-	-	1489.6	1692.4	-	-	1388.4	1383.2	-	-
29	4	1542	-	-	1486.0	1540.8	1411.2	1406.8	-	-	1508.0	1485.2	-	-	1447.6	1378.8	-	-
31	5	1644	-	-	1489.2	1627.6	1390.8	1405.6	-	-	1509.2	1541.2	-	-	1374.4	1445.6	-	-
33	5	1660	-	-	1617.6	1689.6	1542.4	1537.2	-	-	1618.0	1619.2	-	-	1558.4	1521.6	-	-
35	5	1636	-	-	1583.6	1657.6	1554.8	1528.4	-	-	1616.0	1603.6	-	-	1545.2	1460.0	-	-

37	5	1651	-	-	1606.4	1677.2	1576.4	1553.2	-	-	1636.8	1629.6	-	-	1547.6	1397.6	-	-
39	5	1703	-	-	1620.8	1680.4	1581.6	1550.0	-	-	1648.0	1633.6	-	-	1541.6	1440.4	-	-
41	5	1674	-	-	1626.4	1706.0	1593.2	1578.0	-	-	1641.6	1631.2	-	-	1549.6	1426.0	-	-

Sulfides (mg/L)

Week	MIW batch	Inf.	Mussel shell Short HRT				Mussel shell Long HRT				Limestone Short HRT				Limestone Long HRT			
			SRBR 1		SRBR 2		SRBR 3		SRBR 4		SRBR 5		SRBR 6		SRBR 7		SRBR 8	
			Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW	Eff.	PW
1	1	35	320	-	90	-	170	-	190	-	70	-	960	-	670	-	980	-
2	1	55	340	-	150	-	150	-	70	-	320	-	180	-	230	-	240	-
3	1	900	235	-	140	-	110	-	50	-	170	-	230	-	230	-	260	-
4	1	625	135	-	90	-	55	-	75	-	115	-	160	-	410	-	415	-
6	2	10	170	280	100	285	35	450	bdl	100	73	530	105	185	113	95	30	105
7	2	25	45	750	50	575	50	165	50	140	100	1625	75	575	60	100	65	95
8	2	15	175	450	55	413	65	630	333	375	55	2052	55	0	105	110	65	140
9	2	14	250	415	190	635	30	565	75	335	190	300	190	1955	90	1095	85	440
10	2	12	170	1005	100	1445	20	294	85	402	260	420	330	182	265	244	105	186
11	2	10	160	30	75	5	45	1870	120	1325	180	20	160	50	255	325	65	1155
12	2	10	350	50	200	50	50	410	100	2415	150	30	135	120	240	505	130	375
13	2	15	220	5	85	10	20	2228	25	400	165	60	60	480	140	265	30	195
14	3	5	240	260	105	410	125	1390	55	1710	95	215	55	340	290	2460	85	805
15	3	0	490	155	115	120	2320	585	35	610	250	110	100	225	180	475	5	1165
16	3	0	200	340	190	185	30	1365	95	950	175	190	45	310	95	360	85	1680
17	3	0	55	208	270	160	20	355	15	320	365	160	135	385	185	330	50	2075
18	3	0	25	75	140	35	20	325	15	185	415	25	115	140	155	90	0	455
19	3	10	220	170	130	205	100	2075	30	500	30	270	10	170	30	470	35	1935
20	3	12	10	30	115	20	20	1395	50	340	<0	10	<0	45	5	65	10	105
22	4	0	-	-	195	5340	925	12400	-	-	400	>16000	-	-	20	9100	-	-
24	4	12	-	-	345	>16000	70	4540	-	-	240	5980	-	-	30	5120	-	-
27	4	6	-	-	180	3220	270	>16000	-	-	360	2340	-	-	380	10440	-	-

29	4	8	-	-	440	>16000	455	13460	-	-	240	12780	-	-	75	14540	-	-
31	5	10	-	-	650	11120	1307	11380	-	-	100	11100	-	-	240	>16000	-	-
33	5	20	-	-	600	7760	1860	10460	-	-	30	16000	-	-	765	7900	-	-
35	5	15	-	-	915	7700	195	9080	-	-	40	14920	-	-	15	4820	-	-
37	5	15	-	-	1610	5000	1450	6260	-	-	30	5040	-	-	35	3580	-	-
39	5	20	-	-	1805	3500	485	7860	-	-	25	>16000	-	-	35	3900	-	-
41	5	12	-	-	1215	3280	905	11420	-	-	30	>16000	-	-	15	6920	-	-

Dissolved metals - Influent (mg/L)

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	21.709	240.190	15.184	182.209	13.481	25.916	0.928	0.096	3.724	0.0027	0.012
7	2	36.498	190.229	9.977	320.459	12.214	9.703	0.810	0.557	3.130	0.0023	0.028
8	2	24.262	146.459	11.235	186.573	13.514	11.417	0.911	0.290	2.933	0.0021	0.026
9	2	23.094	137.869	10.615	209.130	13.379	11.503	0.858	0.832	3.558	0.0028	0.059
10	2	22.980	148.329	15.733	184.020	14.044	15.332	1.001	0.320	4.208	0.0028	0.024
11	2	22.747	145.699	14.829	180.061	15.087	13.388	1.034	0.179	4.258	0.0028	0.016
12	2	22.794	144.286	15.355	181.826	14.110	12.936	0.977	0.138	3.424	0.0026	0.014
13	2	20.670	125.314	11.805	199.701	14.123	9.855	0.972	0.502	3.709	0.0026	0.041
14	3	10.936	155.290	20.437	183.753	18.441	60.471	1.133	0.113	4.766	0.0028	0.009
15	3	10.506	148.874	19.617	182.015	17.874	53.430	1.174	0.391	4.897	0.0028	0.023
16	3	13.288	182.215	22.905	201.182	18.195	55.311	1.187	0.191	4.713	0.0027	0.011
17	3	8.834	120.537	14.985	150.759	16.802	45.293	1.059	0.166	4.939	0.0031	0.012
18	3	11.385	162.515	18.879	171.504	18.303	48.844	1.141	0.136	5.116	0.0030	0.010
19	3	10.275	151.436	18.685	174.846	18.124	44.176	1.101	0.159	5.125	0.0030	0.011
20	3	8.937	132.384	15.214	165.519	17.063	38.745	1.519	1.112	20.141	0.0103	0.181
22	4	6.979	112.620	14.558	125.790	15.094	35.624	0.864	0.187	3.918	0.0033	0.011
24	4	7.397	124.883	15.626	156.296	15.920	31.482	0.892	0.101	3.495	0.0021	0.008
27	4	6.828	115.994	13.988	144.611	15.164	22.896	0.827	0.125	3.913	0.0021	0.009
29	4	7.643	126.813	15.065	144.435	15.662	21.752	0.946	0.156	4.347	0.0025	0.008
31	5	12.254	139.046	15.174	161.171	16.085	28.427	0.963	0.133	4.425	0.0037	0.011
33	5	8.706	110.707	11.925	149.014	15.807	20.196	1.064	0.118	4.533	0.0031	0.010
35	5	7.731	98.565	10.789	133.254	14.306	15.228	0.960	0.091	4.064	0.0028	0.009
37	5	13.307	173.200	18.422	173.423	16.244	15.449	0.958	0.110	4.822	0.0030	0.010
39	5	14.461	187.509	20.057	195.105	17.754	15.811	1.041	0.110	5.188	0.0032	0.010
41	5	10.606	136.778	15.776	163.378	17.725	15.918	1.116	0.095	4.485	0.0031	0.009

Total metals - Influent (mg/L)

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	26.418	290.120	18.951	220.678	15.620	32.110	1.049	0.124	3.857	0.003	0.014
7	2	34.042	187.652	14.855	318.854	15.256	23.798	0.959	0.704	3.251	0.003	0.045
8	2	20.294	136.201	10.567	217.259	13.825	14.176	0.942	0.291	2.961	0.002	0.037
9	2	21.141	125.650	9.637	204.793	13.272	13.085	0.843	0.790	3.522	0.003	0.070
10	2	24.047	151.387	16.127	189.383	14.718	31.970	1.023	0.333	3.960	0.003	0.026
11	2	23.539	150.124	15.401	188.301	15.530	15.417	1.072	0.179	4.010	0.003	0.017
12	2	22.903	145.867	15.734	187.074	14.126	13.502	0.968	0.138	3.139	0.003	0.015
13	2	23.620	141.809	13.831	221.000	15.505	33.859	1.053	0.567	3.802	0.003	0.045
14	3	12.007	172.693	22.814	205.509	20.374	73.873	1.233	0.097	5.054	0.003	0.010
15	3	13.417	188.299	24.714	215.205	20.709	67.575	1.257	0.171	4.966	0.003	0.011
16	3	14.827	203.905	26.614	224.900	21.044	61.278	1.282	0.246	4.877	0.003	0.013
17	3	9.034	125.013	15.458	153.432	17.074	45.907	1.090	0.157	4.650	0.003	0.013
18	3	11.772	164.507	19.562	177.214	18.477	49.396	1.143	0.103	4.725	0.003	0.010
19	3	11.796	171.755	21.757	197.632	20.220	49.332	1.221	0.194	4.970	0.003	0.012
20	3	10.349	153.697	18.101	187.359	18.646	52.525	1.637	1.340	19.543	0.011	0.198
22	4	9.173	121.004	15.931	134.966	16.159	37.858	0.905	0.165	3.872	0.003	0.012
24	4	7.069	118.064	14.093	157.200	16.424	31.733	0.930	0.122	3.483	0.002	0.009
27	4	7.085	118.438	14.231	153.520	16.282	24.903	0.880	0.131	3.638	0.002	0.010
29	4	8.416	134.832	15.955	150.155	17.178	24.035	1.027	0.140	3.885	0.003	0.009
31	5	12.847	147.917	16.580	173.778	16.877	31.880	1.026	0.206	3.936	0.004	0.014
33	5	8.696	110.259	11.957	151.695	15.977	20.484	1.062	0.098	4.173	0.003	0.010
35	5	8.538	109.939	11.913	146.944	15.772	17.773	1.048	0.099	4.206	0.003	0.010
37	5	15.238	199.777	21.401	204.243	18.858	17.800	1.262	0.107	4.505	0.003	0.010
39	5	16.475	216.231	23.147	218.533	20.010	17.983	1.122	0.118	4.732	0.003	0.011
41	5	10.995	140.602	16.242	173.851	18.649	16.996	1.169	0.103	4.299	0.003	0.010

Dissolved metals - Effluent (µg/L) - SRBR 1

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	20976.819	119881.135	52.310	247130.956	3755.627	331.378	22.863	5.833	8.429	0.068	0.607
7	2	27848.805	131681.592	43.438	326097.058	8313.661	809.837	34.859	19.195	8.311	0.069	0.334
8	2	21537.595	118708.942	45.966	273080.145	8785.980	1345.974	55.677	19.354	12.018	0.149	0.578
9	2	20971.719	115229.413	54.440	250700.807	9620.658	1526.883	133.986	25.498	28.547	<0.000	0.575
10	2	25428.234	145968.361	121.671	295809.249	11108.997	1876.569	199.174	31.232	19.144	0.036	1.071
11	2	22894.953	132167.648	85.428	273883.744	10921.780	1405.088	164.916	48.453	11.337	0.009	1.158
12	2	22937.758	132805.061	112.231	276242.914	10557.262	2542.843	185.760	28.243	8.720	0.012	0.566
13	2	22354.216	126103.292	85.450	301766.210	10909.254	960.011	112.320	19.742	17.738	0.021	0.691
14	3	18696.889	169797.287	272.155	341098.110	14462.515	3407.281	298.450	12.277	30.431	0.117	0.380
15	3	14187.298	157167.437	520.717	334575.189	15070.479	4143.677	380.970	14.911	95.911	0.033	0.637
16	3	15788.374	180356.184	666.594	342814.643	15711.186	4154.402	417.689	22.574	705.081	0.024	0.516
17	3	10926.468	126367.587	462.064	271114.142	16196.567	5263.644	404.867	44.352	702.460	0.101	0.795
18	3	12751.899	162996.858	3570.998	276049.174	17154.137	10503.890	353.958	8.620	2387.740	0.071	0.435
19	3	13136.481	161678.310	277.126	334820.687	16910.838	5988.017	314.655	32.796	24.052	<0.000	1.016
20	3	10412.668	126691.495	81.423	298117.691	14701.395	2161.025	369.947	31.716	575.973	0.051	0.651

Dissolved metals - Effluent (µg/L) - SRBR 2

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	24226.851	114138.575	42.313	263805.939	4860.566	3352.653	308.794	15.437	32.308	0.040	0.598
7	2	27261.512	124314.455	27.386	344521.384	6724.023	618.655	14.632	41.024	9.892	0.060	0.334

8	2	27102.414	138783.577	37.548	278870.512	7915.398	739.264	26.262	26.728	13.447	0.074	0.977
9	2	20559.518	110310.390	27.110	266516.558	7844.316	909.106	69.250	38.613	13.650	<0.000	0.672
10	2	23519.835	130288.115	39.027	294320.636	8274.443	825.188	51.191	39.361	18.217	0.041	0.864
11	2	23058.121	129878.884	38.543	299005.666	8519.773	1088.165	32.461	22.365	10.057	0.024	0.613
12	2	23060.046	135653.903	96.455	287743.543	9772.620	1322.789	151.259	32.497	27.430	0.022	0.842
13	2	21742.566	120222.956	33.628	302376.590	8962.290	625.093	48.496	28.160	9.801	0.009	0.568
14	3	20425.558	155331.443	76.771	367445.534	10297.257	1515.686	90.689	3.778	22.467	0.077	0.282
15	3	13743.147	153100.058	30.316	372148.440	11474.923	713.673	61.239	15.349	9.940	0.008	0.377
16	3	18051.008	197391.449	35.135	421677.140	13931.673	987.585	78.758	28.928	24.145	0.004	0.474
17	3	12094.636	135719.797	236.161	336312.402	14944.001	1219.174	93.498	24.396	87.009	0.082	1.031
18	3	13660.330	171189.218	1941.782	320673.909	18889.833	7016.187	67.513	19.670	1327.807	0.127	1.944
19	3	12601.733	152348.373	25.586	357406.363	15165.957	1190.397	59.398	16.153	8.370	0.030	0.489
20	3	11289.175	134271.037	24.766	342550.275	14000.616	1542.941	79.365	20.089	14.176	<0.000	0.685
22	4	10493.466	136079.029	60.148	281382.146	13454.088	2816.080	136.152	50.286	80.558	0.045	1.448
24	4	8678.360	121759.519	54.064	292489.950	12447.880	2315.701	128.044	15.805	18.558	0.208	0.896
27	4	11271.303	161641.612	47.966	347356.140	15405.376	2505.593	183.294	41.277	41.840	0.048	1.208
29	4	9154.280	125333.388	119.313	254138.507	14519.363	3419.329	303.192	65.140	26.590	0.348	0.797
31	5	12911.822	152236.822	217.483	289866.861	14748.470	3176.131	270.186	33.437	22.998	0.134	0.546
33	5	12545.027	143288.156	267.011	289845.234	15879.835	2387.265	387.179	92.228	100.294	0.145	1.367
35	5	8949.163	102514.884	143.163	226327.006	13329.883	2019.007	311.664	46.789	21.331	0.000	0.525
37	5	16173.109	190494.268	360.056	306757.737	15942.646	2932.493	354.415	94.702	67.044	0.059	1.185
39	5	16577.185	195410.417	410.244	320301.158	16416.978	3012.101	434.445	26.904	12.924	0.026	0.732

41	5	14700.138	176989.537	621.978	290405.866	17474.550	3316.277	495.640	23.501	11.199	0.040	0.593

Dissolved metals - Effluent (µg/L) - SRBR 3

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	27201.453	97632.758	67.780	228743.543	2719.181	583.959	89.248	19.822	15.009	0.054	0.809
7	2	33676.556	81516.178	63.744	318268.630	3062.532	687.471	22.474	45.603	7.231	0.143	0.401
8	2	28272.414	116229.067	35.370	301775.425	5372.961	950.430	27.178	17.905	11.599	0.116	0.758
9	2	22031.490	96203.981	26.551	292889.618	4969.583	517.817	60.486	11.274	9.804	<0.000	0.347
10	2	24978.136	115555.773	32.594	323258.882	5326.501	613.512	96.236	43.549	14.399	0.016	0.694
11	2	23921.690	120133.564	34.496	321224.657	6322.138	451.882	22.492	46.527	28.014	0.010	0.931
12	2	23521.728	117685.492	34.376	319509.775	5791.592	789.659	78.208	17.616	8.465	0.023	0.501
13	2	22824.046	109078.695	26.467	328768.623	5614.576	389.031	10.729	18.794	15.342	0.019	0.653
14	3	25189.514	124820.861	35.746	364295.701	5726.027	921.249	14.711	3.552	9.114	0.077	0.234
15	3	17764.205	129423.758	28.688	371780.782	6638.145	333.615	3.038	8.425	4.443	0.005	0.471
16	3	19579.772	174428.597	24.529	425229.156	9646.552	506.941	284.801	21.509	171.492	0.006	0.493
17	3	11873.148	123113.357	20.090	320015.304	11830.179	1496.346	350.275	31.746	315.932	0.149	0.553
18	3	13928.679	154519.119	37.726	352179.693	12988.615	2998.499	214.697	7.743	8.997	0.011	0.494
19	3	13486.166	144231.068	13.374	372900.445	10665.092	733.924	220.420	35.219	173.866	0.008	0.701
20	3	12001.043	126794.892	10.688	348850.351	9304.824	327.374	45.540	33.729	32.782	<0.000	0.720
22	4	11031.552	125599.446	52.752	258066.340	10384.524	2681.774	37.903	25.438	23.179	0.058	1.450
24	4	9541.413	120223.113	47.447	305694.009	11309.210	1232.410	58.434	6.469	11.111	0.018	0.304
27	4	10848.684	144048.206	62.960	334512.218	11356.739	1565.318	53.015	43.771	22.544	0.062	1.483

29	4	9911.787	125602.120	43.559	283235.943	11070.985	1107.702	82.819	31.380	6.761	0.052	0.527
31	5	11652.986	136311.705	50.787	307152.601	9849.977	580.229	27.646	26.518	13.521	0.228	0.960
33	5	10801.989	124397.906	43.519	301122.504	10655.167	511.361	32.217	23.515	20.811	0.180	0.805
35	5	8988.256	97552.039	24.533	250308.807	9270.081	215.837	13.645	33.074	4.415	0.000	0.507
37	5	16072.838	175482.662	55.714	339690.623	11153.503	313.989	11.161	13.191	15.289	0.365	0.311
39	5	17224.547	191233.025	67.596	375340.700	12421.012	423.339	14.088	52.055	19.638	1.677	0.847
41	5	14981.291	164138.603	95.668	335299.459	14813.053	881.379	45.616	18.024	12.004	0.030	0.549

Dissolved metals - Effluent (µg/L) - SRBR 4

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	23578.209	89880.273	64.912	198023.708	2595.307	730.952	16.545	17.280	10.488	0.321	0.630
7	2	34050.966	87314.834	63.638	309620.217	3578.401	719.061	10.446	10.939	6.546	0.073	0.200
8	2	25156.594	100455.867	44.319	301258.674	5348.632	913.322	30.260	17.020	9.211	0.070	0.722
9	2	22696.115	99766.425	31.829	280559.979	5956.496	686.641	20.963	18.484	5.996	0.003	0.446
10	2	26436.413	123954.087	50.652	330949.303	7127.475	863.347	22.483	17.204	16.276	0.016	0.978
11	2	24172.641	117866.499	41.542	312616.093	7204.309	518.723	21.541	27.341	6.948	0.016	0.649
12	2	24125.244	119595.882	51.654	308444.283	6737.438	1226.767	37.612	22.968	30.423	0.020	0.704
13	2	24431.636	117366.410	36.412	330200.028	7787.399	549.118	17.267	23.238	9.869	0.013	0.610
14	3	23987.214	122772.552	41.703	348081.772	6771.862	358.667	19.037	25.407	38.055	0.077	0.402
15	3	18065.603	133222.105	37.279	374590.868	8546.503	371.208	13.361	20.529	30.730	0.016	0.549
16	3	16584.424	177393.389	596.075	350042.826	14571.327	5236.948	385.384	5.752	20.552	0.003	0.354
17	3	12403.937	122272.780	32.039	307523.464	12377.789	4074.144	269.847	40.273	155.626	0.095	0.507

18	3	15097.450	172956.479	65.274	385797.492	15695.250	3529.087	219.203	14.737	10.531	0.019	0.613
19	3	13841.587	141761.773	35.527	367878.881	10559.990	237.103	315.021	30.745	181.342	0.029	0.648
20	3	12617.867	125663.191	22.209	356303.836	9146.917	223.162	44.241	31.531	80.053	<0.000	0.809

Dissolved metals - Effluent (µg/L) - SRBR 5

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	19740.729	136716.592	54.146	257465.036	4502.272	697.834	60.918	20.741	33.554	0.072	0.743
7	2	25108.804	133544.881	47.167	303723.594	9321.101	2179.855	141.344	50.936	19.975	0.293	0.592
8	2	21936.527	127333.396	59.344	258897.127	10018.660	2278.844	82.000	44.616	33.767	0.132	0.961
9	2	17951.595	103819.270	96.244	217790.294	9364.865	2081.653	134.474	56.841	12.730	0.003	0.923
10	2	22805.422	135798.958	201.157	267940.478	10874.367	2410.674	163.734	13.333	9.162	0.005	0.666
11	2	24331.384	148946.142	232.460	294831.866	12613.829	2288.627	220.103	20.536	15.046	0.018	0.679
12	2	20847.128	128548.837	140.854	254925.701	10083.015	2415.067	152.237	75.471	94.136	0.048	1.854
13	2	21781.740	129979.075	99.118	291986.632	11097.542	1487.107	102.869	13.109	9.121	0.011	0.572
14	3	17373.548	153060.432	248.643	318650.770	13466.500	4099.002	140.664	7.114	28.255	0.067	0.325
15	3	12776.231	159195.106	411.221	329472.154	15390.605	2930.926	277.917	19.573	246.419	0.011	0.540
16	3	13431.007	179888.379	1693.549	304801.094	16252.642	5340.687	431.392	12.238	943.788	0.039	0.510
17	3	9919.114	134408.796	986.284	266615.270	16845.741	5301.278	405.679	41.061	464.085	0.065	0.791
18	3	11348.381	162074.502	3349.517	273315.419	17901.623	6243.510	719.011	21.438	2075.690	0.008	0.596
19	3	10900.164	154380.153	208.391	320962.319	16804.573	1625.465	394.028	45.542	838.669	0.241	0.955
20	3	9625.860	137070.502	245.382	295042.798	16024.060	163.527	426.176	65.729	1457.716	0.844	0.882
22	4	7532.991	116974.128	238.536	209561.961	13380.347	7045.109	246.543	61.125	302.751	0.063	1.445

24	4	6861.541	112666.471	639.091	239851.045	14118.855	7286.048	381.818	22.425	576.738	0.055	0.885
27	4	8534.569	143709.140	1217.046	264928.929	15793.942	7177.605	376.172	53.806	478.437	0.068	1.540
29	4	7583.479	121621.979	4237.017	208224.988	14751.684	209.298	470.111	124.191	1600.313	0.617	7.011
31	5	11007.047	142276.053	1143.902	241396.614	15034.585	6045.554	356.183	80.395	370.460	0.138	1.598
33	5	9283.152	115878.662	4473.131	212360.579	15035.175	436.987	581.189	103.339	1731.984	0.663	13.863
35	5	7672.197	99337.750	1834.672	197712.075	13302.393	3419.274	447.034	107.061	1344.434	0.600	8.476
37	5	12883.799	168763.655	9621.401	227061.003	15180.961	2538.939	711.310	109.601	3137.328	1.482	26.541
39	5	13735.357	184548.925	13448.005	248304.219	16314.157	938.259	835.257	101.261	2750.047	1.023	23.245
41	5	14153.815	176967.497	3529.662	294051.644	19973.865	2949.974	658.918	84.913	1778.226	0.542	4.706

Dissolved metals - Effluent (µg/L) - SRBR 6

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	19135.056	136258.349	53.935	255241.560	4626.515	881.118	55.750	11.630	10.471	0.042	0.762
7	2	24765.892	131597.776	46.617	290090.490	9407.600	2424.748	84.703	30.270	34.126	0.072	0.353
8	2	21735.345	128286.631	59.884	278914.856	10301.425	2970.983	114.575	42.013	14.580	0.244	1.014
9	2	19094.141	111340.318	83.599	228234.317	9840.818	2359.779	124.822	30.312	29.847	0.007	0.714
10	2	24147.627	145676.436	280.483	274024.127	11875.396	2939.738	258.284	32.978	41.863	0.024	0.938
11	2	21141.750	130550.580	150.376	255184.992	11144.949	2011.869	170.892	28.114	11.645	0.014	0.782
12	2	21527.311	134714.207	102.341	271500.416	11110.335	3488.239	111.658	21.990	18.802	0.017	0.699
13	2	21212.894	125300.826	73.240	287770.698	11523.602	1489.544	64.193	62.866	12.728	0.025	1.257
14	3	17123.000	157243.586	159.528	325995.677	14318.856	3614.266	106.053	21.317	58.408	0.056	0.606
15	3	12663.381	161361.791	177.264	332260.999	16329.505	5076.245	151.279	14.538	36.586	0.010	0.487

16	3	13725.630	181716.288	1320.533	324240.692	17615.049	6541.355	484.635	9.641	899.824	0.064	1.090
17	3	10624.108	137031.171	204.327	289656.566	17962.970	6923.010	268.269	33.940	196.987	0.063	0.721
18	3	12211.956	163432.971	901.314	319686.425	15353.652	6229.641	273.708	16.737	132.193	0.066	1.378
19	3	11020.082	154624.582	86.296	324411.951	17221.053	1301.694	367.262	44.327	421.586	0.108	0.661
20	3	10079.167	145102.562	493.346	302195.400	17205.932	144.711	507.932	47.043	1163.164	0.336	1.042

Dissolved metals - Effluent (µg/L) - SRBR 7

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	24184.289	83178.907	83.415	167204.658	3021.848	692.660	211.884	29.279	160.528	0.098	0.914
7	2	31301.156	53709.412	101.718	172965.804	2975.330	660.701	121.010	35.376	259.839	0.115	0.788
8	2	25756.891	105387.710	46.977	224875.651	6641.376	1624.592	133.670	74.482	42.303	0.088	1.485
9	2	-	-	-	-	-	-	-	-	-	-	-
10	2	26559.315	118500.159	37.543	316372.330	6927.973	907.082	122.951	27.362	93.525	0.011	0.782
11	2	23555.680	124825.493	31.887	293434.184	8246.164	738.134	138.184	31.881	88.831	0.018	0.936
12	2	22956.042	123140.583	69.705	280425.841	8354.608	1298.956	175.136	21.811	262.583	0.041	0.522
13	2	22260.180	108237.753	33.009	296094.999	6873.827	445.805	110.503	40.257	640.025	0.036	0.839
14	3	21071.050	128335.286	38.660	331751.527	8132.695	868.115	41.687	11.708	16.120	0.031	0.379
15	3	15676.966	151646.604	77.877	347818.621	12344.027	2856.718	112.061	20.174	116.110	0.017	0.519
16	3	16135.145	169427.672	185.669	343550.526	13389.378	4080.802	226.558	25.598	307.930	0.007	0.526
17	3	11497.957	130233.463	169.427	286745.849	14429.107	2947.784	271.743	23.053	211.351	0.081	0.514
18	3	12614.024	160422.713	1034.829	299283.594	15335.017	8867.937	313.847	16.919	119.456	0.074	1.705
19	3	12197.100	148566.842	46.871	310679.719	13345.840	2173.621	168.258	48.097	708.467	0.344	0.874

20	3	11048.119	132708.061	29.611	312427.686	11778.559	571.467	177.867	39.960	264.905	0.034	0.806
22	4	8822.382	112958.258	39.957	230497.842	10031.783	666.819	104.693	58.697	71.032	0.063	1.286
24	4	7376.105	108276.872	43.581	251044.557	11206.569	598.034	97.112	18.883	24.633	0.014	0.340
27	4	7798.456	126226.730	238.135	246581.187	12289.908	3624.048	186.847	64.226	78.978	0.054	1.372
29	4	7692.267	121717.227	826.185	207515.629	13211.996	4917.234	358.497	53.918	545.036	0.093	1.250
31	5	9625.562	134058.826	156.094	258226.145	11806.542	3335.349	74.369	27.706	15.500	0.074	0.785
33	5	8657.883	112072.883	261.635	226274.414	13184.069	4745.306	300.096	80.651	168.103	0.027	1.767
35	5	7331.904	93473.903	307.827	192120.991	11470.438	1245.499	320.916	53.620	773.044	0.403	2.043
37	5	13214.694	173861.352	1115.111	243779.581	14022.513	3176.583	460.829	51.829	1034.584	0.845	2.439
39	5	14012.986	183038.443	1656.783	259943.891	15036.816	4893.027	431.417	15.275	293.176	0.054	1.671
41	5	11920.883	153693.768	608.778	243928.796	15847.867	1669.177	449.895	91.319	578.192	0.194	2.237

Dissolved metals - Effluent (µg/L) - SRBR 8

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	-	-	-	-	-	-	-	-	-	-	-
7	2	30519.338	54663.998	94.566	180360.065	3278.177	934.736	300.450	24.956	73.662	0.047	0.318
8	2	23265.959	95716.108	119.085	225489.981	5688.639	1425.785	42.628	14.686	19.210	0.126	0.824
9	2	19032.558	92834.242	18.533	236563.528	6875.564	434.119	170.050	37.750	650.661	0.031	0.819
10	2	24372.383	113748.561	40.027	294917.925	6854.452	1197.178	64.642	11.196	131.179	0.014	0.438
11	2	23203.357	122481.224	44.552	284643.234	9134.282	1394.912	84.962	6.008	10.879	0.013	0.374
12	2	23362.043	129532.836	67.481	284634.672	9310.760	2045.271	185.829	3.427	78.159	0.016	0.338
13	2	24294.456	117692.765	28.009	319603.048	8045.190	716.611	82.722	10.877	62.748	0.006	0.361

14	3	20590.946	121347.157	44.342	314440.083	7336.846	449.356	107.523	10.316	107.605	0.035	0.332
15	3	14177.416	146531.959	472.068	311312.109	13854.820	7166.189	243.474	36.953	442.020	0.323	1.290
16	3	15868.159	168197.096	213.002	337951.046	13474.101	6174.800	183.200	7.923	58.194	0.001	0.382
17	3	11291.226	128126.890	131.522	289460.445	14194.466	6488.045	235.330	32.759	109.390	0.114	0.631
18	3	13466.539	162270.570	206.396	336011.096	13610.672	5436.442	236.897	5.352	25.474	0.031	0.593
19	3	12203.763	156328.679	190.411	309304.937	14861.431	6375.458	246.939	55.252	597.275	0.097	1.015
20	3	10553.149	138325.014	251.164	287201.377	13360.361	4714.960	323.552	32.049	517.580	0.028	0.693

Total metals - Effluent (µg/L) - SRBR 1

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	20976.82	119881.14	52.31	247130.96	719.515	101.663	5.490	14.722	16.254	0.080	0.481
7	2	27848.81	131681.59	43.44	326097.06	1625.477	203.611	7.345	5.899	17.160	0.073	0.303
8	2	21537.60	118708.94	45.97	31874.853	1671.854	400.199	30.779	6.547	79.349	0.073	0.710
9	2	20971.72	115229.41	54.44	250700.81	1825.867	355.952	27.398	11.384	77.440	0.056	1.143
10	2	25428.23	145968.36	121.67	295809.25	1968.724	485.186	37.444	26.764	109.832	0.066	1.191
11	2	22894.95	132167.65	85.43	273883.74	2122.294	557.593	39.264	19.349	184.951	0.105	1.322
12	2	22937.76	132805.06	112.23	276242.91	1972.643	671.819	40.863	9.933	115.286	0.082	0.794
13	2	22354.22	126103.29	85.45	301766.21	1771.563	232.752	22.346	14.166	80.859	0.047	1.843
14	3	18696.89	169797.29	272.16	341098.11	2804.134	763.512	57.621	6.581	106.494	0.035	0.686
15	3	14187.30	157167.44	520.72	334575.19	2802.516	1027.149	69.483	9.761	208.287	0.083	1.124
16	3	15788.37	180356.18	666.59	342814.64	3154.247	1523.032	81.382	30.510	231.436	0.136	2.046
17	3	10926.47	126367.59	462.06	271114.14	2576.432	1193.296	70.261	13.232	150.375	0.095	1.536

18	3	12751.90	162996.86	3571.00	276049.17	2705.686	1210.029	52.462	19.313	111.107	0.048	1.450
19	3	13136.48	161678.31	277.13	334820.69	2923.222	1289.629	56.023	18.900	81.032	0.033	0.947
20	3	10412.67	126691.50	81.42	298117.69	2990.073	768.424	74.635	20.348	112.139	0.193	0.574

Total metals - Effluent (µg/L) - SRBR 2

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	4785.127	22535.356	28.856	51482.248	926.377	1077.273	59.514	14.152	27.654	0.066	0.632
7	2	5506.253	25077.209	45.518	70159.928	1347.112	166.786	3.063	6.641	20.134	0.021	0.869
8	2	3334.747	18932.774	73.810	49570.572	1208.331	149.747	4.660	10.623	38.551	0.025	0.623
9	2	3778.268	20370.936	86.773	50861.637	1477.127	224.415	13.399	6.356	41.800	0.045	1.183
10	2	4889.420	26967.617	145.863	62592.395	1748.460	276.541	11.097	13.339	42.320	0.029	0.622
11	2	4478.780	25742.492	704.088	57920.760	1661.902	636.086	10.014	21.406	214.996	0.134	2.410
12	2	4175.065	24688.670	342.207	53144.272	1775.305	398.458	28.864	7.444	78.419	0.056	0.676
13	2	4344.722	24293.386	187.457	61511.679	1794.635	232.425	11.024	6.643	55.311	0.041	0.653
14	3	3842.522	28248.635	176.761	70152.128	2000.878	423.424	17.976	6.836	54.160	0.056	0.346
15	3	2616.020	28521.591	211.755	68943.518	2110.572	263.634	11.857	3.132	49.749	0.021	0.986
16	3	3068.792	35090.328	150.820	76651.169	2537.360	268.743	15.025	16.084	43.529	0.016	0.765
17	3	1915.200	21933.767	265.236	55319.132	2211.807	317.659	16.545	5.887	96.840	0.056	0.741
18	3	2376.521	28101.043	279.312	61996.544	2580.373	374.106	17.450	7.783	70.915	0.030	0.502
19	3	2485.813	30091.435	178.446	69448.945	2925.337	380.846	12.048	7.604	41.085	0.016	0.320
20	3	2320.781	27515.503	216.451	68265.238	2730.192	455.399	17.562	16.881	72.955	0.035	0.553
22	4	1862.978	21340.761	300.938	48051.091	2327.184	1071.164	24.358	10.415	94.029	0.058	0.909

24	4	1466.086	20556.631	403.360	53418.610	2170.352	690.324	23.511	5.616	91.154	0.059	0.562
27	4	1585.303	22612.530	333.570	52431.745	2408.083	597.221	30.649	10.183	71.442	0.036	0.677
29	4	1810.129	24669.659	771.719	48755.670	2690.465	762.259	57.605	12.771	154.443	0.062	1.394
31	5	1973.541	23432.892	1103.324	47752.676	2471.297	686.256	47.934	15.730	199.495	0.093	2.289
33	5	1652.679	18899.112	791.007	42174.973	2453.693	527.280	64.754	9.486	220.460	0.111	1.946
35	5	1587.124	17628.309	920.719	39033.559	2286.112	456.688	60.049	10.977	261.300	0.135	2.134
37	5	3030.483	36049.313	2781.438	58645.552	2994.244	719.058	83.688	24.559	581.817	0.259	5.226
39	5	3021.175	35221.205	2532.654	58049.708	2931.930	645.293	86.104	24.082	392.517	0.201	4.519
41	5	1998.228	24037.559	1158.066	42712.103	2743.103	660.020	80.784	25.507	265.448	0.187	3.059

Total metals - Effluent (µg/L) - SRBR 3

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	5194.322	18890.228	22.285	44022.553	508.630	136.235	16.621	5.146	11.612	0.046	0.305
7	2	6102.842	14862.640	16.674	58876.521	561.180	155.728	4.262	5.336	7.355	0.029	0.430
8	2	3542.662	16190.656	11.671	51821.711	843.007	159.815	6.559	1.841	7.178	0.010	0.150
9	2	4030.568	17781.828	12.045	55251.203	950.973	110.535	11.397	5.955	9.664	0.013	0.337
10	2	5000.357	22737.081	17.998	65622.128	1078.752	138.150	20.292	13.448	12.266	0.019	0.200
11	2	4282.678	21307.093	22.053	57063.798	1101.922	95.634	4.199	4.201	6.647	0.009	0.135
12	2	4481.248	22550.487	67.669	61518.334	1113.695	208.789	17.529	4.000	27.569	0.019	0.316
13	2	4346.398	20861.777	7.855	63145.610	1059.613	92.868	2.471	4.117	2.683	0.001	0.141
14	3	4464.987	22573.003	13.200	67704.020	1086.804	257.978	4.704	5.902	12.086	0.019	0.133
15	3	3440.866	24371.003	21.311	69200.250	1251.531	111.171	0.967	1.841	11.761	0.021	1.454

16	3	3453.027	31278.721	14.241	77807.147	1748.684	147.521	52.356	7.420	38.248	0.004	0.175
17	3	1922.440	20040.075	12.308	52792.490	1819.424	492.325	93.362	3.060	59.669	0.029	0.267
18	3	2442.061	25936.614	19.244	63237.128	1932.243	207.024	10.352	3.871	19.328	0.006	0.191
19	3	2621.110	28270.989	24.214	72484.700	2089.646	325.009	62.014	14.244	56.319	0.016	0.481
20	3	2684.075	28229.768	12.633	74632.577	1944.630	151.822	9.763	6.032	10.391	<0.000	0.170
22	4	1992.049	20983.634	76.237	45528.716	1834.522	525.793	7.842	8.738	28.657	0.481	0.588
24	4	1689.836	21382.980	59.818	54316.062	1988.569	272.280	10.626	3.642	19.616	0.017	0.363
27	4	1602.798	21048.379	142.714	51664.914	1829.034	308.366	10.563	7.827	33.935	0.025	0.515
29	4	1863.544	24165.908	52.743	51742.605	2009.382	225.467	23.386	14.106	16.670	0.030	0.271
31	5	1901.573	22530.952	29.774	52093.724	1661.068	135.831	7.335	10.882	8.317	0.013	0.457
33	5	1691.636	18694.533	47.281	48566.700	1752.160	101.693	7.749	2.092	14.448	0.007	0.242
35	5	1626.964	17925.016	27.247	47742.710	1707.072	48.075	3.343	7.290	7.494	0.000	0.147
37	5	2884.967	31861.244	45.111	61566.851	2041.707	70.897	2.687	3.544	8.345	0.007	0.145
39	5	3118.898	34357.357	61.381	69059.313	2217.945	100.952	3.110	5.615	10.545	0.007	0.138
41	5	2144.407	24263.345	77.002	51479.405	2362.192	156.697	11.447	17.055	16.105	0.010	0.313

Total metals - Effluent (µg/L) - SRBR 4

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	5097.059	19300.240	29.549	42902.835	557.894	203.753	3.847	8.047	6.881	0.051	0.335
7	2	6260.936	15881.352	20.569	58578.146	676.900	156.591	2.347	4.252	6.432	0.143	0.555
8	2	3745.607	16557.994	23.111	50469.653	952.429	207.844	6.207	3.759	12.342	0.087	0.220
9	2	4256.884	18189.315	42.870	54093.248	1147.672	162.919	4.473	4.180	22.488	0.011	0.236

10	2	5117.736	23555.848	74.369	64259.833	1325.319	184.011	5.266	7.495	23.100	0.033	0.243
11	2	4836.015	23934.392	109.777	62706.739	1445.159	140.331	5.877	12.853	35.715	0.022	0.359
12	2	4523.920	21635.108	127.152	58477.118	1247.519	286.711	10.770	4.739	42.581	0.027	0.263
13	2	4158.375	19629.794	33.421	57103.543	1323.200	132.406	4.019	5.997	12.396	0.009	0.166
14	3	4399.276	22685.130	165.400	66586.883	1310.002	154.739	4.549	6.428	64.673	0.040	0.428
15	3	3361.315	24521.461	35.779	69589.233	1543.417	106.431	2.721	2.059	21.673	0.009	0.169
16	3	2994.223	33303.002	961.027	65782.237	2773.245	2335.361	75.276	19.634	203.530	0.110	1.030
17	3	2055.267	20116.823	21.090	51690.152	1889.292	892.509	45.895	6.422	29.555	0.031	0.172
18	3	2485.070	26807.340	32.678	61233.241	2174.622	311.350	9.386	10.027	10.184	0.004	0.186
19	3	2744.331	28287.383	118.870	71555.485	2031.216	437.725	289.379	15.854	40.138	0.015	0.435
20	3	2443.840	26168.232	134.045	69578.741	2093.389	363.416	40.878	17.457	43.951	0.019	0.769

Total metals - Effluent (µg/L) - SRBR 5

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	3900.264	25654.777	39.784	49704.354	853.755	173.868	11.521	10.763	14.772	0.069	0.400
7	2	4616.338	25024.870	99.290	58101.973	1721.607	461.045	26.625	6.799	51.957	0.094	0.633
8	2	3158.231	20321.375	112.523	44631.354	1774.527	460.485	15.491	10.690	38.481	0.038	0.611
9	2	3645.678	21189.520	149.193	44722.642	1899.537	501.613	27.910	4.352	44.448	0.025	0.569
10	2	4497.140	26964.406	279.170	53798.420	2083.177	544.658	31.760	14.594	63.626	0.054	0.528
11	2	3915.046	24078.650	472.769	46566.260	2076.467	535.477	36.987	10.893	125.002	0.075	0.970
12	2	4201.427	25792.216	342.591	51494.188	2015.256	607.308	31.289	6.474	76.875	0.055	0.644
13	2	3896.064	22774.502	179.710	52170.062	2009.268	336.658	21.068	8.587	61.239	0.073	0.812

14	3	3249.952	28512.507	151.784	60719.457	2597.168	827.447	27.174	8.048	36.116	0.026	0.381
15	3	2276.672	28424.465	547.233	58123.150	2721.169	946.381	48.939	11.735	146.832	0.095	1.106
16	3	2529.694	33339.547	1663.339	58581.639	3093.026	2191.678	80.834	42.790	252.091	0.226	2.947
17	3	1590.549	21124.619	540.050	43334.804	2608.454	1186.705	68.854	10.410	187.955	0.119	1.196
18	3	2032.581	28213.311	907.577	52394.157	2826.288	1483.296	56.080	15.240	203.001	0.117	1.400
19	3	2284.127	32272.600	438.216	68303.271	3466.135	1391.091	80.686	17.286	171.063	0.094	1.033
20	3	1938.249	27726.646	1067.756	58414.980	3037.760	2909.415	80.613	45.740	251.934	0.162	2.736
22	4	1384.088	21120.979	1008.273	38309.791	2435.926	3240.561	44.767	31.159	241.575	0.228	3.115
24	4	1272.582	21240.377	632.206	44112.017	2550.695	1874.280	68.664	11.417	164.646	0.086	0.977
27	4	1291.787	21308.228	1543.701	42195.577	2571.633	1785.732	64.216	29.351	456.923	0.200	4.069
29	4	1445.750	23298.963	883.329	39075.227	2689.267	1186.176	84.271	20.140	239.076	0.122	2.076
31	5	1847.159	23840.229	430.852	41638.660	2570.175	1189.242	60.297	12.426	79.951	0.213	1.179
33	5	1624.588	20482.919	808.240	38722.154	2702.338	784.641	106.032	22.285	290.153	0.115	3.244
35	5	1444.326	18519.332	693.796	35509.558	2381.024	1052.631	80.019	15.673	228.909	0.123	2.268
37	5	2603.316	33906.176	1938.557	45243.415	3031.446	969.654	138.093	41.142	499.454	0.223	4.900
39	5	2549.952	33675.169	2549.493	45635.977	2960.141	1271.739	147.876	32.120	468.241	0.166	4.382
41	5	1928.403	25242.360	710.506	44790.739	2986.422	736.274	97.229	11.925	245.701	0.089	1.110

Total metals - Effluent (µg/L) - SRBR 6

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	4073.999	28559.262	61.844	52622.838	932.352	241.783	11.857	8.219	20.753	0.732	0.417
7	2	4461.589	24479.938	81.146	54024.927	1724.139	485.389	15.649	5.834	21.631	0.042	0.402

8	2	2993.235	19186.793	127.988	41199.072	1751.878	582.240	19.442	10.120	49.000	0.199	0.719
9	2	3681.922	21698.404	136.543	46186.532	1880.925	524.529	24.987	5.712	41.136	0.021	0.494
10	2	4550.824	27129.262	690.813	52984.445	2231.326	908.095	48.033	20.203	175.551	0.100	1.434
11	2	3862.097	23848.693	560.808	46895.349	2024.282	604.476	34.729	15.775	142.038	0.096	1.304
12	2	4016.589	25074.018	227.115	51093.398	2085.720	812.726	21.507	14.534	51.770	0.034	0.575
13	2	3705.997	21464.812	121.366	50488.764	2032.962	366.895	11.812	5.232	30.331	0.023	0.299
14	3	3189.291	29450.513	95.173	62371.706	2795.848	752.272	20.117	7.408	24.660	0.009	0.200
15	3	2455.234	29354.779	254.587	59431.604	2864.535	1044.151	28.364	32.195	136.073	1.429	0.339
16	3	2521.758	34154.345	695.824	61104.432	3300.319	1693.740	91.329	14.996	188.296	0.067	2.057
17	3	1612.037	20894.803	258.879	45427.319	2569.853	1484.493	43.070	4.535	78.983	0.076	1.460
18	3	2139.457	29413.614	1046.742	52494.832	3060.383	1974.733	71.552	12.018	228.079	0.124	3.457
19	3	2125.738	30137.360	244.508	62525.495	3221.011	875.461	69.154	16.916	78.103	0.033	0.773
20	3	2003.804	28065.489	203.805	63843.749	2960.266	1739.445	37.740	24.103	85.496	0.042	1.520

Total metals - Effluent (µg/L) - SRBR 7

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	4776.711	16619.047	30.963	33267.468	581.815	200.235	40.548	11.710	61.555	0.113	0.469
7	2	4833.029	8343.805	24.840	28801.572	486.535	141.180	19.628	6.657	50.756	0.034	0.327
8	2	3670.015	16287.919	25.447	48258.163	1113.602	307.312	23.196	5.544	34.703	0.049	0.281
9	2	-	-	-	-	-	-	-	-	-	-	-
10	2	5154.360	23185.271	24.675	61953.647	1303.927	217.156	23.297	21.402	80.808	0.054	0.592
11	2	4096.325	21420.111	31.370	51500.454	1449.962	217.721	24.813	12.038	101.205	0.037	0.294

12	2	4190.790	23198.932	101.934	52177.321	1535.091	389.825	32.722	17.805	130.918	0.071	0.446
13	2	4017.629	19285.327	20.123	52382.190	1256.352	180.766	20.957	20.508	198.733	0.181	0.357
14	3	4129.807	24050.856	24.214	63955.230	1560.718	201.962	8.449	15.786	80.906	0.106	0.319
15	3	2815.078	26359.498	62.648	62077.552	2136.830	602.776	19.661	9.239	110.320	0.104	0.350
16	3	2902.395	31928.720	81.880	64606.886	2504.157	856.504	43.030	27.811	90.468	0.108	0.746
17	3	1752.560	20039.160	82.003	44730.667	2139.466	554.763	44.780	26.978	125.376	0.191	1.347
18	3	2366.814	28726.706	134.279	55802.671	2512.223	838.682	47.113	18.970	76.578	0.051	0.691
19	3	2301.108	28127.437	50.059	59054.620	2503.278	675.769	31.412	17.549	174.540	0.097	0.468
20	3	2196.749	26932.975	30.431	60290.638	2252.865	296.397	33.844	17.180	48.232	0.028	0.314
22	4	1865.448	21316.614	26.853	42387.533	1853.377	341.198	19.461	6.557	18.188	0.155	0.541
24	4	1394.835	20853.759	20.221	46601.940	2077.932	195.910	17.699	4.809	8.760	0.019	0.273
27	4	1343.890	21086.569	1653.199	42843.521	2196.638	1866.980	36.888	23.670	259.812	0.187	3.380
29	4	1494.070	23095.557	518.227	39400.118	2452.839	1062.894	65.468	11.563	106.254	0.043	0.860
31	5	1636.753	22786.467	68.844	45504.317	2044.022	595.901	13.410	8.297	11.352	0.012	0.467
33	5	1428.755	18322.368	1810.888	37364.517	2237.243	1824.112	61.740	86.300	303.300	0.282	2.015
35	5	1422.107	17967.276	132.241	35686.301	2128.893	308.933	59.577	7.073	134.017	0.182	0.695
37	5	2529.185	32578.373	529.567	46429.482	2614.501	710.737	84.493	14.086	163.642	0.054	1.036
39	5	2654.045	34793.896	601.458	49051.755	2835.643	1025.844	79.542	7.453	49.985	0.012	0.892
41	5	1902.804	24468.244	165.575	40534.871	2676.269	288.384	74.552	32.673	88.196	0.036	0.715

Total metals - Effluent (µg/L) - SRBR 8

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	4654.479	16158.595	30.521	33060.257	585.981	278.750	34.642	21.618	123.036	0.306	0.650
7	2	4645.687	8453.702	21.524	29180.029	522.920	222.246	49.243	6.833	16.235	0.027	0.326
8	2	3320.457	15029.415	14.324	45413.054	970.566	294.757	7.994	21.770	38.602	0.091	0.704
9	2	3893.052	18720.341	22.846	48597.886	1416.478	250.474	34.725	5.001	141.765	0.015	0.415
10	2	4808.874	22448.754	24.961	58166.742	1343.174	283.416	12.548	17.347	66.347	0.055	0.487
11	2	3867.953	20860.633	86.418	48288.419	1567.964	576.240	15.206	42.106	78.849	0.108	2.106
12	2	4202.090	22897.247	196.322	51245.691	1696.532	550.821	33.847	12.740	58.415	0.050	0.618
13	2	4261.739	20424.585	16.208	56288.460	1444.109	292.036	15.522	6.145	23.425	0.029	0.274
14	3	3954.427	23340.414	46.108	61570.967	1447.146	287.561	21.782	10.526	52.240	0.038	0.985
15	3	2662.195	28243.232	52.331	60408.331	2602.748	1389.363	42.984	3.520	50.307	0.045	1.360
16	3	2899.423	31171.836	152.412	63546.552	2530.439	1350.490	34.820	14.707	52.618	0.023	0.836
17	3	1746.003	19372.494	85.452	44927.927	2013.839	1133.907	38.109	5.117	32.470	0.052	0.587
18	3	2387.312	28543.392	100.840	56237.154	2497.669	1183.076	32.671	9.292	24.220	0.014	0.329
19	3	2564.655	31021.308	1393.229	69846.756	2535.792	1748.027	478.905	51.468	478.602	0.224	4.485
20	3	2199.758	28069.971	153.021	58696.924	2645.598	1113.694	63.149	15.234	92.765	0.027	0.412

Dissolved metals - Pore-water (µg/L) - SRBR 1

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	4781.665	44810.558	15.519	62856.799	2069.710	615.453	159.626	3.557	2.058	0.010	0.132
7	2	5946.262	31204.395	32.602	66155.837	2368.768	464.770	107.359	4.550	3.875	0.007	0.049

8	2	4350.916	24937.333	14.999	54235.554	2143.476	460.003	79.623	9.083	5.628	0.026	0.249
9	2	3956.764	22772.121	15.306	47746.617	2044.685	424.894	86.533	1.704	2.722	0.000	0.066
10	2	4914.488	28878.239	48.627	54064.030	2448.620	469.236	374.422	0.941	9.216	0.002	0.058
11	2	4445.639	26905.999	161.923	48072.084	2600.339	497.645	487.543	7.405	306.754	0.003	0.208
12	2	4289.057	26621.890	609.807	45896.950	2430.015	625.044	280.520	9.032	378.202	0.060	0.630
13	2	4536.821	27192.772	374.527	49857.442	2922.037	1199.262	475.193	11.489	532.891	0.008	0.231
14	3	2832.824	31939.923	142.937	64978.936	3217.680	823.990	127.622	0.667	115.123	0.004	0.043
15	3	2723.994	33822.987	429.546	64319.941	3499.744	867.735	184.885	2.811	378.265	0.002	0.122
16	3	3251.992	38235.857	155.862	77687.108	3440.549	952.601	102.097	6.698	21.157	0.016	0.192
17	3	2275.816	25828.347	84.610	57191.075	3368.056	1366.428	167.265	3.094	34.392	0.025	0.190
18	3	3053.421	36748.758	35.261	69792.921	3365.207	1178.989	66.897	7.435	111.862	0.016	0.175
19	3	2735.199	36720.102	1267.044	61160.242	3824.079	1832.185	338.032	9.014	688.082	0.042	0.609
20	3	2898.183	32478.148	9.649	78969.825	3059.730	1810.085	153.975	3.765	19.453	0.013	0.163

Dissolved metals - Pore-water (µg/L) - SRBR 2

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	4711.357	40573.737	6.942	65444.952	1591.835	495.831	43.960	3.438	2.723	0.008	0.339
7	2	5788.678	28409.335	8.532	70478.065	1783.389	360.136	69.380	1.820	3.005	0.005	0.050
8	2	4118.231	25432.086	9.895	57884.859	1819.582	315.038	44.659	5.768	2.062	0.015	0.290
9	2	4076.581	22941.230	10.595	51512.565	1956.090	300.064	55.499	2.203	1.118	0.000	0.069
10	2	4840.155	28440.839	44.636	57269.389	2234.398	427.079	151.172	8.792	11.474	0.006	0.241
11	2	4327.416	26130.129	34.273	49472.729	2503.110	449.457	238.326	1.067	54.957	0.001	0.073

12	2	4543.147	26868.485	213.484	56706.656	2163.036	310.436	50.194	8.772	107.509	0.038	0.696
13	2	4549.078	26653.332	30.210	55413.875	2655.344	365.295	201.713	6.756	44.167	0.001	0.164
14	3	2955.340	31723.929	75.300	68323.536	3197.959	682.667	91.079	3.671	10.393	0.007	0.125
15	3	2753.357	33657.018	37.032	71842.932	3386.506	612.492	55.288	1.852	2.121	0.001	0.079
16	3	2993.427	37538.334	539.598	64556.174	3854.812	1422.097	195.279	2.779	554.114	0.005	0.137
17	3	2076.916	25365.183	704.266	50352.169	3807.622	2205.718	237.451	19.982	559.887	0.107	1.366
18	3	3008.898	35928.389	55.931	75271.986	3148.704	312.627	24.800	15.431	30.639	0.034	1.853
19	3	2559.459	33079.653	316.990	59063.457	3760.306	1268.730	201.510	3.377	444.675	0.010	0.209
20	3	2589.626	30252.850	37.786	74923.542	3432.441	1681.979	39.590	2.029	2.360	0.012	0.149
22	4	1698.669	23238.190	33.945	45764.469	2927.571	2555.328	94.257	3.294	16.836	0.022	0.417
24	4	1528.137	22534.009	459.171	49395.791	3032.845	2524.111	118.700	9.731	147.548	0.130	1.254
27	4	1776.260	25511.871	83.476	51801.195	3223.787	2216.893	102.091	11.162	56.356	0.122	0.259
29	4	1747.183	23427.005	1023.387	48707.929	3055.624	3051.999	166.073	30.334	393.729	0.208	2.591
31	5	2516.162	28234.330	1060.581	53599.172	3173.584	2295.281	119.853	18.479	289.506	0.185	1.910
33	5	1924.736	22147.242	383.048	44986.412	3016.484	2092.494	142.499	5.785	180.795	0.001	0.152
35	5	1780.618	20345.431	121.754	43681.083	2813.938	1195.542	107.949	6.442	4.374	0.007	0.141
37	5	3137.298	37925.964	1443.398	56191.622	3531.395	2041.015	163.931	7.916	461.198	0.007	0.139
39	5	3153.110	38156.869	2378.361	56613.935	3541.203	2067.968	167.637	4.697	587.205	0.005	0.117
41	5	2646.396	31059.416	1885.339	52945.754	3801.069	1643.531	161.569	2.889	437.641	0.009	0.132

Dissolved metals - Pore-water (µg/L) - SRBR 3

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	5290.844	23060.541	9.175	53384.523	632.610	115.758	165.659	1.208	3.594	0.009	0.123
7	2	5988.917	26607.196	9.692	74494.568	1428.284	312.618	185.413	1.912	1.727	0.009	0.071
8	2	4030.316	21787.651	5.405	60287.119	1321.751	116.896	8.148	3.248	1.807	0.016	0.241
9	2	4130.491	20909.181	4.627	56379.861	1333.065	103.268	7.960	0.876	0.470	0.000	0.069
10	2	5018.907	26871.678	5.821	66639.937	1625.826	96.637	119.409	2.207	1.224	0.000	0.132
11	2	4685.047	25317.185	7.319	62353.824	1666.389	90.388	176.398	3.501	1.549	0.001	0.114
12	2	4489.134	24797.940	6.291	60431.871	1529.739	111.348	8.669	1.112	1.058	0.002	0.095
13	2	4080.114	22275.820	4.935	58689.410	1597.266	87.779	162.185	1.202	2.080	0.002	0.100
14	3	3584.563	27142.276	6.239	71293.175	2019.430	164.069	19.419	2.180	8.256	0.005	0.066
15	3	3117.258	32297.075	10.701	79273.493	2573.140	311.366	10.107	1.551	4.228	0.003	0.105
16	3	3277.578	34289.868	25.101	79514.164	2452.288	443.044	9.845	1.819	10.234	0.006	0.135
17	3	2303.761	24091.377	8.499	64865.555	2726.422	937.768	75.835	2.724	7.626	0.017	0.083
18	3	2978.466	31976.174	4.205	75048.108	2303.177	111.085	12.380	8.314	13.640	0.012	0.172
19	3	2866.005	31782.553	5.036	76359.726	2479.841	253.361	67.543	1.337	1.594	0.001	0.098
20	3	2465.767	26368.938	4.260	72888.134	2125.482	124.157	4.712	1.351	1.581	0.008	0.139
22	4	2090.879	23401.756	8.772	54032.139	1838.797	124.149	17.133	3.623	4.182	0.015	0.246
24	4	1666.333	20795.620	6.968	55075.088	1900.407	145.917	3.958	0.623	1.615	0.001	0.063
27	4	1888.004	24168.266	67.591	62084.364	2009.963	149.166	3.179	4.453	20.722	0.021	0.375
29	4	2019.174	24841.592	9.451	59287.863	2062.544	92.515	2.953	1.167	1.595	0.005	0.138
31	5	2343.962	26554.427	9.794	62106.832	1983.596	84.009	2.174	1.604	2.253	0.005	0.149

33	5	2098.401	22701.674	11.711	60062.887	2100.675	79.454	4.849	1.234	3.692	0.004	0.125
35	5	1889.777	20353.022	792.363	55128.542	2006.247	883.354	36.391	7.094	318.567	0.169	2.108
37	5	3254.734	35933.746	1619.844	71601.605	2375.759	829.096	51.978	11.167	471.000	0.176	2.420
39	5	3486.669	38322.585	513.491	75376.945	2500.243	427.011	19.464	3.846	140.502	0.050	0.773
41	5	2590.954	28504.547	44.309	64022.187	2696.943	114.677	6.818	1.832	12.305	0.027	0.159

Dissolved metals - Pore-water (µg/L) - SRBR 4

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	5587.704	30409.099	9.768	61086.114	957.299	178.866	15.630	1.164	1.840	0.007	0.099
7	2	6210.019	27876.986	14.018	74568.355	1610.660	339.578	29.100	1.666	1.895	0.075	0.023
8	2	3954.964	22178.892	6.743	57382.909	1451.847	120.085	30.442	4.486	1.510	0.028	0.215
9	2	4089.937	21118.327	5.301	55302.224	1464.898	107.582	7.701	1.083	2.794	0.000	0.077
10	2	5428.329	29251.906	7.695	69209.523	1899.431	141.871	112.850	2.131	1.856	0.001	0.129
11	2	4737.310	26621.502	13.596	60982.721	1878.874	124.437	103.763	2.706	1.501	0.002	0.347
12	2	4446.025	24768.670	7.801	58651.325	1614.842	113.791	11.022	0.588	0.940	0.001	0.058
13	2	4450.019	24473.716	324.785	61450.484	1986.360	220.167	120.916	4.298	99.479	0.063	0.856
14	3	3687.102	28004.000	8.938	71973.277	2125.107	133.251	28.412	1.383	2.511	0.003	0.099
15	3	3236.748	32137.766	180.396	78685.341	2463.344	396.011	16.452	2.307	51.497	0.036	0.342
16	3	3113.963	34517.567	15.851	73299.325	2855.262	732.435	48.492	3.022	3.360	0.003	0.098
17	3	2281.489	24949.113	7.830	61462.300	3092.123	934.231	176.043	4.225	2.923	0.018	0.088
18	3	3143.520	33673.564	8.783	76726.818	2665.484	237.991	11.730	6.526	7.290	0.006	0.146
19	3	3414.774	36517.458	8.843	88575.789	3130.630	296.268	142.058	1.669	2.168	0.003	0.127

20	3	2359.180	24819.622	4.888	68419.172	2135.771	123.653	30.793	1.620	1.531	0.007	0.138

Dissolved metals - Pore-water (µg/L) - SRBR 5

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	4197.049	43518.113	54.238	54871.706	2101.659	598.556	124.774	4.402	3.054	0.009	0.124
7	2	5473.579	31258.622	22.501	63444.518	2474.342	628.072	226.909	3.560	3.685	0.004	0.053
8	2	3482.034	23652.017	35.356	45493.041	2282.058	489.666	106.821	9.455	2.753	0.013	0.258
9	2	3844.232	23167.797	36.164	47618.520	2337.552	408.944	82.060	3.040	5.357	0.000	0.076
10	2	4932.329	30259.797	101.518	53261.345	2766.960	455.509	493.068	7.263	143.077	0.001	0.258
11	2	4109.454	26379.227	384.873	44795.601	2588.546	340.175	380.578	7.226	439.457	0.031	0.318
12	2	4254.566	26834.583	62.704	50664.737	2387.519	400.927	57.074	2.669	0.995	0.001	0.168
13	2	4204.810	25132.452	138.283	49570.912	2749.161	274.656	427.997	6.767	202.249	0.009	0.228
14	3	2693.651	31906.346	762.664	62259.884	3328.232	1167.947	103.017	13.207	170.900	0.115	1.438
15	3	2438.967	34010.466	254.312	65306.571	3733.544	830.869	113.554	2.205	121.343	0.008	0.114
16	3	2606.515	35556.185	926.119	62675.970	3547.114	1283.652	147.425	11.666	427.809	0.078	0.769
17	3	1876.179	25187.410	582.857	48262.843	3717.990	831.681	442.176	6.548	531.639	0.025	0.558
18	3	2424.700	33789.802	137.742	60525.925	3344.385	1125.796	67.985	5.204	63.361	0.011	0.182
19	3	2162.671	31034.837	347.444	55853.294	3659.980	985.903	356.346	8.752	459.486	0.006	0.160
20	3	1862.617	26726.510	63.058	61271.252	3228.006	1401.063	69.370	4.385	3.672	0.014	0.164
22	4	1496.779	22491.773	27.806	44295.852	2746.334	1989.511	64.438	11.017	13.242	0.028	0.270
24	4	1276.874	21376.087	10.648	46880.440	2723.543	1499.575	100.073	2.023	7.152	0.001	0.161
27	4	1454.605	24274.233	83.345	52647.828	3112.555	2135.109	107.630	4.863	33.798	0.021	0.347

29	4	1602.541	25340.658	94.272	49631.992	2943.805	1410.652	38.953	3.858	6.482	0.007	0.150
31	5	2164.065	28323.525	1469.301	50482.850	3003.270	2032.964	112.592	26.065	432.737	0.249	2.724
33	5	1793.100	22510.224	34.706	49328.262	2843.843	1007.289	51.417	5.365	3.709	0.005	0.237
35	5	1585.853	19892.357	30.486	44428.111	2553.878	793.909	26.454	4.801	0.987	0.000	0.112
37	5	2580.195	33245.026	741.632	50895.388	2873.552	1147.665	106.473	11.874	231.669	0.108	1.130
39	5	2893.895	36809.414	2240.548	62885.528	3171.049	1553.567	64.419	18.024	624.770	0.320	3.318
41	5	2225.971	28764.836	69.302	53857.562	3237.417	863.347	44.709	6.192	13.992	0.010	0.213

Dissolved metals - Pore-water (µg/L) - SRBR 6

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	4908.814	50317.382	27.384	68105.471	2209.471	752.821	82.877	1.366	1.561	0.007	0.097
7	2	5009.828	28474.951	28.699	57183.449	2195.738	624.688	231.988	7.303	2.436	0.002	0.079
8	2	3523.251	23407.297	28.971	47533.633	2143.614	560.981	57.906	4.843	2.188	0.015	0.205
9	2	3989.560	23660.250	34.626	49960.852	2218.612	491.069	53.113	1.755	0.643	0.008	0.072
10	2	5080.644	30963.459	83.098	58582.916	2608.451	552.852	86.000	8.844	3.022	0.003	0.196
11	2	4221.881	26700.803	57.847	49287.763	2417.692	387.997	100.681	6.173	17.496	0.001	0.136
12	2	4317.748	26562.583	49.110	54187.422	2176.550	582.715	27.359	2.572	2.761	0.004	0.094
13	2	4247.324	25270.718	39.760	55357.711	2496.280	459.383	63.698	4.333	3.966	0.002	0.160
14	3	2894.289	31790.089	96.775	64894.416	3257.896	1035.370	74.835	1.388	18.320	0.010	0.156
15	3	-	-	-	-	-	-	-	-	-	-	-
16	3	2613.587	33330.228	27.999	69118.985	2832.261	1070.868	39.662	0.683	2.025	0.003	0.077
17	3	2069.380	24961.859	16.303	56729.376	2998.719	1008.668	48.216	3.042	2.284	0.035	0.106

18	3	2316.097	32055.406	196.775	56475.569	3342.900	1216.918	80.715	1.268	168.515	0.008	0.133
19	3	2148.946	30432.294	11.067	65992.180	3059.714	1200.586	67.751	4.642	2.093	0.000	0.159
20	3	1980.742	27720.885	21.732	64774.960	3110.453	1632.020	34.915	3.410	2.267	0.017	0.175

Dissolved metals - Pore-water (µg/L) - SRBR 7

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	6090.320	18191.616	14.461	40476.046	558.857	232.442	202.291	4.178	5.445	0.011	0.284
7	2	6401.306	21765.361	9.381	66048.821	1150.471	482.350	219.645	1.895	2.226	0.006	0.070
8	2	3847.500	19800.755	6.609	53121.259	1342.948	366.587	15.891	1.366	2.094	0.017	0.134
9	2	4058.247	19874.325	5.960	53318.515	1354.203	207.647	9.194	0.977	0.720	0.000	0.119
10	2	4897.679	25395.893	8.376	62417.809	1601.258	298.057	32.939	1.429	1.783	0.001	0.109
11	2	4435.406	24890.906	11.665	57124.144	1808.173	401.501	56.598	2.404	1.614	0.001	0.117
12	2	4315.112	24640.601	11.997	55780.346	1688.326	391.846	8.151	3.527	1.260	0.002	0.095
13	2	4391.700	23254.380	10.165	59931.050	1807.548	224.942	40.511	0.850	3.262	0.003	0.095
14	3	3151.928	30246.450	33.101	67639.669	2625.147	1233.394	22.705	1.658	3.160	0.003	0.112
15	3	2805.477	32259.058	42.853	68420.419	2928.298	1548.239	28.407	1.641	3.286	0.007	0.088
16	3	2742.762	33696.246	2302.912	65324.516	2957.515	3239.015	64.879	35.727	417.528	0.285	5.540
17	3	2053.142	25510.344	178.804	53589.287	3272.067	2024.304	110.855	5.864	25.769	0.027	0.493
18	3	2646.092	32142.404	58.985	59730.764	2815.862	868.279	53.858	2.320	33.922	0.014	0.111
19	3	2294.356	30054.827	34.048	61400.652	3025.262	1964.325	80.127	3.539	1.714	0.001	0.137
20	3	2024.313	26143.483	12.804	61776.409	2428.359	796.162	7.793	1.649	1.022	0.006	0.124
22	4	1759.431	22924.455	94.051	48095.284	2072.797	550.816	14.326	3.878	34.168	0.020	0.485

24	4	1467.946	21487.600	12.990	51108.953	2139.174	403.331	8.428	1.221	1.237	0.002	0.073
27	4	1499.385	23528.591	323.878	51496.004	2347.849	676.941	23.590	8.371	108.582	0.052	0.992
29	4	1702.096	25821.732	4.938	52037.264	2364.167	556.184	47.318	5.238	1.244	0.014	0.106
31	5	1904.542	26013.407	39.637	53393.149	2335.163	785.859	18.805	8.983	12.916	0.011	0.200
33	5	1702.351	21415.894	25.104	49946.757	2405.512	602.464	16.185	2.799	3.072	0.006	0.235
35	5	1599.632	20152.447	43.510	46914.766	2177.660	347.415	9.743	3.094	10.936	0.005	0.182
37	5	2717.042	34533.426	29.995	58299.087	2366.682	277.397	10.965	2.734	2.757	0.005	0.082
39	5	2810.833	35315.116	31.129	62090.265	2297.095	191.295	13.509	2.320	2.057	0.003	0.097
41	5	2246.434	28197.480	93.762	50576.098	2740.413	595.549	48.497	1.654	1.307	0.005	0.133

Dissolved metals - Pore-water (µg/L) - SRBR 8

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	5318.049	16822.718	16.767	36703.253	537.395	169.366	198.445	1.870	9.530	0.006	0.231
7	2	6613.836	23243.187	11.765	70217.887	1271.735	396.208	447.155	1.052	3.019	0.004	0.070
8	2	3652.403	19868.749	9.356	51119.829	1441.017	468.520	58.691	4.517	1.906	0.018	0.210
9	2	3897.069	19904.715	7.622	50882.310	1512.692	284.172	34.024	1.211	1.530	0.000	0.084
10	2	4774.367	25044.145	10.551	60221.011	1607.726	308.147	260.004	0.999	5.510	0.003	0.094
11	2	4749.595	26360.410	12.202	61100.577	1892.868	347.509	403.346	0.876	1.875	0.002	0.080
12	2	4282.605	24343.825	27.836	54385.223	1715.534	345.978	18.915	2.568	4.072	0.004	0.121
13	2	4352.187	22766.131	8.839	60139.697	1736.250	154.088	362.704	0.855	2.048	0.004	0.160
14	3	3366.443	30065.433	41.055	68012.747	2716.494	1309.633	50.612	1.002	3.113	0.041	0.072
15	3	2693.437	28532.728	64.177	64246.717	2465.878	1139.334	22.446	1.321	13.217	0.012	0.137

16	3	3074.848	32997.040	23.202	69602.382	2439.198	937.866	32.099	1.121	2.174	0.005	0.116
17	3	2336.424	23929.113	26.090	61120.517	2396.591	555.678	346.777	3.834	7.580	0.012	0.143
18	3	2602.808	31454.093	34.724	60951.251	2768.119	1196.266	37.265	6.379	17.148	0.010	0.151
19	3	2525.834	30305.916	12.584	66977.805	2481.442	610.968	287.647	2.576	1.341	0.001	0.113
20	3	2098.816	25687.489	10.532	63895.315	2255.170	483.551	51.795	1.333	2.648	0.004	0.119

Total metals - Pore-water (µg/L) - SRBR 1

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	5317.258	49509.135	803.147	68106.944	2186.066	1295.077	169.731	28.871	209.561	0.168	1.827
7	2	4646.445	25013.562	4915.868	55563.104	1971.150	2540.221	150.641	41.297	1219.935	0.860	7.005
8	2	3072.065	19491.094	4009.748	42971.179	1812.332	2234.873	132.424	45.043	1202.034	1.011	9.509
9	2	3915.486	21761.862	8639.584	47873.736	2026.897	4624.158	196.933	96.582	2321.090	2.101	19.330
10	2	4703.111	27344.773	16196.398	53667.654	2357.475	7804.226	454.584	179.748	3001.485	2.845	26.413
11	2	4330.422	26905.321	22081.719	48175.836	2623.631	10830.926	619.384	264.477	4262.080	4.267	38.409
12	2	3857.654	23413.813	17633.062	43159.060	2205.606	8443.608	402.716	185.705	2756.351	2.922	25.982
13	2	4252.054	25237.536	49845.725	49300.911	2757.417	33065.820	1243.961	560.817	10298.855	9.290	77.326
14	3	2532.417	27338.980	7173.857	58653.129	3009.581	8116.652	279.897	90.947	1687.929	1.168	10.245
15	3	2366.691	28974.811	13574.267	58218.462	3167.265	41052.906	420.851	168.848	2742.039	2.099	22.284
16	3	2864.450	34151.165	27199.114	71802.572	3176.858	33497.482	736.438	313.375	5035.482	3.622	31.321
17	3	2523.147	29695.533	18452.992	58862.195	3044.262	31527.215	453.640	244.502	3249.994	2.188	25.646
18	3	2270.926	28573.653	17921.631	50518.613	3097.923	27557.119	544.976	276.246	4074.423	2.900	31.769
19	3	2368.170	31260.423	10423.840	54801.370	3506.664	13425.617	503.883	190.367	2531.735	1.567	18.815

20	3	2506.242	27921.401	3683.239	73363.292	2901.333	7037.553	288.352	141.306	1038.327	0.628	13.940

Total metals - Pore-water (µg/L) - SRBR 2

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	5104.027	43787.763	489.403	71411.879	1639.093	1046.502	53.662	39.199	192.988	0.154	2.624
7	2	4666.391	23405.371	1937.495	59421.604	1508.912	1100.207	105.036	32.481	451.049	0.328	3.430
8	2	3108.398	18966.076	1557.341	44822.296	1472.387	952.749	94.131	52.583	509.794	0.380	6.543
9	2	3598.451	19976.984	4481.399	45702.177	1712.432	2479.982	114.816	110.343	1329.017	1.100	13.855
10	2	4556.883	26128.601	11614.759	54861.135	2159.285	6054.725	255.543	234.023	2707.860	2.230	24.977
11	2	4085.697	24535.127	20343.734	47289.051	2438.370	9794.727	432.790	395.174	4780.808	4.274	51.585
12	2	4037.475	23995.821	5448.556	52641.663	1943.630	2049.282	119.967	132.982	1126.217	0.928	11.566
13	2	4117.468	24126.507	18520.252	49802.655	2410.835	8362.271	307.481	381.739	4016.189	3.810	41.315
14	3	2480.037	27695.361	6844.278	60789.145	2891.128	6328.677	211.847	140.755	1575.808	1.170	13.278
15	3	2399.827	28848.209	13388.289	67684.346	3244.083	14650.463	427.475	251.432	3179.102	2.272	26.284
16	3	2713.379	35182.381	32952.440	61384.340	3713.543	48976.430	553.507	476.599	4447.796	3.252	42.256
17	3	2214.356	26873.634	19091.630	50797.502	3345.447	21050.864	483.359	320.680	3737.790	2.502	29.239
18	3	2208.261	26990.238	5359.081	53059.490	3073.959	6913.720	233.109	91.159	1527.611	0.885	9.782
19	3	2244.551	28576.195	15119.665	53690.450	3472.164	14230.182	386.530	262.854	3072.342	2.014	25.236
20	3	2430.949	27831.487	3329.295	71421.088	3228.232	4534.985	178.380	68.971	958.445	0.571	6.074
22	4	1449.791	20357.031	13740.831	40929.808	2700.368	22412.498	649.920	313.984	3929.573	2.551	28.613
24	4	1363.138	20462.523	19406.612	45837.707	2796.189	18741.074	450.140	362.115	5203.889	3.616	49.965
27	4	1381.487	19855.647	8968.453	43394.384	2706.744	8438.937	276.781	145.563	2623.415	1.650	21.858

29	4	1556.970	21591.958	14636.975	42849.796	2757.973	16002.614	544.197	291.125	4171.063	2.771	39.288
31	5	2098.333	23798.745	9733.077	45926.755	2691.426	8067.124	311.348	140.285	2463.438	1.652	22.600
33	5	1627.177	18349.464	6582.913	38668.096	2685.953	7150.910	297.956	122.736	2484.311	1.744	23.540
35	5	1579.127	17653.418	4443.278	38908.355	2458.602	4224.756	217.788	80.050	1868.463	1.246	19.830
37	5	2750.437	33432.705	8949.292	50141.804	3048.799	7127.224	270.479	96.691	2336.084	1.548	21.550
39	5	2919.364	34784.670	6902.584	51449.929	3209.742	4101.536	216.277	78.427	1745.545	1.132	15.172
41	5	2007.214	23401.444	3925.796	41292.797	3070.462	3039.043	195.273	54.904	1240.899	0.871	11.250

Total metals - Pore-water (µg/L) - SRBR 3

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	5797.507	25481.213	254.933	59201.616	663.678	434.243	193.041	39.549	60.439	0.088	1.811
7	2	4964.026	21893.494	121.980	63987.209	1198.578	439.049	181.377	16.242	78.470	0.043	1.202
8	2	3327.106	17796.052	120.521	51041.230	1103.345	244.804	12.700	12.719	76.987	0.040	0.883
9	2	4025.650	20284.696	175.434	56050.477	1336.053	237.779	12.929	12.736	68.196	0.047	1.038
10	2	4934.664	26130.637	304.566	66830.824	1599.127	331.221	176.334	14.574	129.700	0.072	1.494
11	2	4284.491	23974.120	356.068	58112.107	1600.509	284.799	210.114	13.495	131.778	0.060	2.293
12	2	4160.776	23077.776	1030.004	56722.179	1442.344	909.739	43.830	16.861	246.394	0.170	2.594
13	2	4262.632	22914.090	305.603	59147.005	1607.154	346.918	237.092	14.947	116.376	0.059	2.317
14	3	3529.715	25658.468	3776.459	86770.555	2220.073	5364.680	184.936	71.772	919.123	0.570	11.364
15	3	3019.093	28186.537	6987.557	99946.957	2717.379	9622.586	303.919	100.433	1891.338	1.098	19.980
16	3	3155.257	32831.051	1944.798	81943.109	2466.810	3174.049	63.492	38.589	456.915	0.245	5.471
17	3	2255.270	24017.592	3894.296	63107.116	2410.692	6271.987	135.721	59.911	932.354	0.621	6.487

18	3	2445.481	26876.468	1515.989	63631.432	2273.529	2129.236	83.975	51.993	600.084	0.260	5.853
19	3	2589.718	28096.880	359.294	69851.500	2259.362	878.958	79.572	50.993	250.715	0.064	2.200
20	3	2397.380	25865.297	44.944	71732.100	2064.652	210.760	5.660	10.661	18.023	0.003	0.350
22	4	1887.231	21408.047	931.023	49122.513	1686.060	1959.074	48.024	30.493	328.578	0.252	2.708
24	4	1649.086	21053.478	2032.000	55717.046	1918.474	2881.558	52.099	33.926	541.017	0.314	5.735
27	4	1542.460	19974.693	2523.161	53885.002	1757.642	2978.928	66.403	37.122	643.949	0.452	5.819
29	4	1744.747	22474.675	2086.021	53835.609	1870.839	2164.617	60.553	28.501	532.467	0.287	4.287
31	5	1949.696	22494.649	3604.258	54369.508	1795.779	3202.437	120.176	45.070	876.602	0.500	7.034
33	5	1696.877	18333.161	1877.499	50852.017	1770.400	1725.502	98.556	27.541	700.057	0.394	5.028
35	5	1736.367	17947.608	2646.036	49040.143	1835.069	3074.173	191.347	31.136	953.597	0.522	7.213
37	5	2958.369	33007.103	5623.289	67513.963	2284.423	3180.104	200.974	55.541	1210.626	0.583	8.989
39	5	3072.827	33758.825	3012.697	69238.166	2330.412	2728.128	129.805	23.978	613.903	0.282	4.549
41	5	2141.363	23677.277	1742.268	53734.267	2363.087	3006.820	139.694	26.889	513.108	0.275	4.285

Total metals - Pore-water (µg/L) - SRBR 4

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	5747.482	31680.495	291.457	65017.449	945.991	558.211	32.540	25.352	67.539	0.093	1.288
7	2	4857.657	22204.699	197.989	60900.032	1307.766	476.472	26.345	9.485	74.062	0.075	0.746
8	2	3267.858	17987.644	116.251	48487.439	1273.266	228.947	34.571	3.530	46.482	0.033	0.427
9	2	4005.427	20341.150	153.287	53721.463	1393.792	208.703	9.964	3.646	53.259	0.034	0.741
10	2	5063.996	27205.424	880.962	65205.811	1754.197	557.554	194.904	45.711	229.689	0.146	1.797
11	2	4381.536	24618.658	1507.956	59060.986	1765.345	678.090	145.205	33.159	419.526	0.254	3.037

12	2	4180.893	23200.020	962.906	55722.463	1512.437	450.357	31.661	27.167	215.607	0.150	1.632
13	2	4181.047	23037.158	1023.623	56514.635	1798.058	466.599	144.951	19.837	291.665	0.172	2.445
14	3	3336.484	25360.717	310.348	65444.523	1958.653	328.273	36.682	9.588	84.147	0.042	0.731
15	3	2778.956	26843.577	2368.093	68698.625	2189.207	1874.261	87.394	37.796	658.343	0.367	4.675
16	3	2982.563	34539.640	13017.337	74382.230	2891.790	10400.668	578.170	179.858	2905.770	1.761	17.296
17	3	2057.028	23060.843	5981.166	57499.961	2570.286	6170.755	318.836	112.106	1760.539	1.177	12.968
18	3	2502.437	28101.089	5838.129	65185.330	2613.276	4925.767	618.083	113.722	1820.387	1.065	14.004
19	3	2730.420	30208.479	411.930	74656.341	2630.960	667.667	147.877	32.994	180.373	0.077	1.951
20	3	2720.032	26842.803	37.656	74709.309	1885.809	319.980	9.747	15.165	29.333	0.014	0.316

Total metals - Pore-water (µg/L) - SRBR 5

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	4785.085	49665.336	1414.675	60378.560	2276.854	2017.457	147.753	33.752	323.427	0.677	3.430
7	2	4016.393	23035.059	5678.617	49476.695	1952.221	3201.739	219.721	88.939	1216.113	1.046	13.727
8	2	2818.189	19373.651	5089.900	38749.129	1968.827	2844.469	152.676	137.140	1660.712	1.527	24.227
9	2	3829.951	23293.018	4326.038	46919.489	2320.698	2585.923	107.866	117.809	1367.928	1.182	16.293
10	2	4404.426	27759.347	14274.403	51392.040	2522.124	7057.219	535.480	275.593	3361.528	2.816	31.727
11	2	3945.141	25030.627	18304.969	43492.905	2585.314	8023.774	501.837	396.510	4456.135	3.933	47.854
12	2	3943.697	24358.006	11293.538	48298.376	2237.764	4554.296	157.764	236.079	2236.886	2.145	24.919
13	2	3820.642	23280.611	12276.611	45401.619	2467.919	4309.378	492.237	287.545	3099.340	2.626	28.044
14	3	2430.002	28640.523	9756.676	59446.751	3129.793	6969.898	198.546	216.086	2274.713	1.778	21.811
15	3	1953.498	27222.216	23401.685	55976.478	3244.921	25676.052	361.434	520.617	5604.095	4.211	51.806

16	3	2407.730	33720.036	30698.251	60665.565	3421.318	34263.858	382.421	594.494	6055.622	4.315	47.111
17	3	1668.882	22303.588	13057.076	43277.714	3034.131	14334.120	476.937	306.111	3037.638	1.989	25.537
18	3	2070.116	29232.304	18325.681	52982.807	3413.981	18793.235	336.910	408.190	4820.339	3.224	37.347
19	3	2016.754	29117.380	10419.607	53801.904	3428.410	11055.673	447.908	278.850	2896.088	1.997	21.257
20	3	2182.095	30683.121	9030.377	70051.720	3531.079	8570.146	288.972	242.630	2447.014	1.599	17.194
22	4	1330.356	20391.967	12134.488	39913.614	2481.544	14319.062	369.217	336.573	3227.365	2.293	25.559
24	4	1236.716	20090.009	26160.358	46983.602	2696.778	28696.615	549.665	567.117	6325.996	5.373	60.449
27	4	1124.603	19254.381	42686.739	64069.633	3373.215	34991.282	1815.683	937.543	13273.057	8.805	107.483
29	4	1418.055	21611.859	6748.958	49517.584	2211.963	7327.880	408.208	137.551	2053.894	1.490	18.703
31	5	2136.088	23219.239	12381.749	46072.909	2698.259	8677.337	378.959	175.408	2968.473	1.910	24.830
33	5	1409.225	17666.957	8440.177	40150.413	2383.696	4697.054	248.526	134.821	3425.890	2.017	23.937
35	5	1501.874	19119.574	2109.094	43178.777	2446.781	1519.671	58.662	38.236	833.732	0.517	5.854
37	5	2638.928	34047.982	31653.026	55155.322	3041.557	7794.951	473.162	219.373	7416.343	4.697	46.108
39	5	2692.000	34577.419	85137.575	71295.862	3467.862	25876.056	1615.213	605.291	18293.341	10.513	96.176
41	5	1838.162	23602.334	37459.987	54312.289	3284.963	17671.037	1101.956	470.278	10774.888	7.321	81.236

Total metals - Pore-water (µg/L) - SRBR 6

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	4695.266	45937.705	787.789	67165.309	1937.306	1071.666	87.053	31.444	153.658	0.217	2.286
7	2	3855.613	21825.768	4632.830	47047.371	1776.197	2388.338	213.429	95.376	1076.607	0.898	15.252
8	2	2892.661	19344.427	921.354	40108.351	1844.924	941.447	57.834	30.156	269.739	0.243	5.149
9	2	3738.460	22378.896	1124.907	46062.306	2081.954	1015.729	54.729	24.556	349.045	0.246	4.078

10	2	4524.275	27529.473	5318.348	53094.848	2387.535	2403.319	92.239	71.076	1365.769	0.939	11.424
11	2	4063.704	25462.733	9247.407	48616.145	2398.424	3987.441	142.682	115.200	2848.564	2.003	24.136
12	2	3999.575	24750.758	333.756	51314.698	2023.144	642.819	28.193	12.056	87.593	0.049	1.059
13	2	3394.151	20463.780	1582.239	43838.588	1995.763	882.055	58.886	26.790	478.354	0.315	3.975
14	3	2619.025	29410.363	3113.640	60332.599	3004.728	2653.132	109.926	37.029	872.586	0.520	6.497
15	3	-	-	-	-	-	-	-	-	-	-	-
16	3	2630.980	33898.044	640.495	69200.471	2923.507	1627.419	52.803	28.574	178.905	0.132	2.857
17	3	1704.286	21127.469	1563.574	50789.048	2404.618	2532.093	59.796	25.246	593.319	0.361	4.274
18	3	1979.959	26862.006	504.618	53333.623	2592.407	1369.702	48.284	10.704	133.631	0.071	1.186
19	3	2081.358	28179.450	370.469	63679.353	2840.202	1407.297	72.937	22.622	174.459	0.088	1.412
20	3	1752.820	25185.867	628.921	53827.193	3006.954	2589.042	89.119	29.039	181.478	0.049	4.816

Total metals - Pore-water (µg/L) - SRBR 7

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	5937.512	17804.686	77.092	40526.334	515.916	351.048	189.333	24.962	25.387	0.099	0.913
7	2	4747.644	16118.132	57.683	51447.159	896.856	517.426	180.894	16.958	26.232	0.086	0.870
8	2	3106.439	16106.352	38.182	44014.219	1144.308	427.203	15.141	5.229	30.660	0.017	0.435
9	2	3929.863	18996.813	30.170	51212.478	1299.729	326.823	10.305	9.663	29.171	0.030	0.554
10	2	4593.540	23710.369	80.525	58331.187	1446.169	379.949	32.640	20.742	62.216	0.024	0.730
11	2	4057.545	22407.948	255.481	53406.690	1696.336	488.119	55.165	30.430	145.611	0.055	1.355
12	2	4083.738	23511.200	341.560	52709.950	1628.183	491.054	10.725	27.150	91.283	0.052	0.935
13	2	3764.303	20211.830	89.809	51334.036	1507.040	284.143	35.729	21.875	79.780	0.035	0.804

14	3	2738.532	26606.786	727.442	60469.800	2381.766	1348.018	23.613	20.182	152.673	0.074	1.254
15	3	2225.257	25628.263	3935.489	59771.628	2524.648	2574.050	49.418	65.728	910.875	0.461	8.407
16	3	2697.467	34041.078	10181.981	67365.304	3016.055	8662.480	102.639	170.945	1679.271	1.262	24.690
17	3	1745.374	21470.708	8799.471	47255.463	2660.999	9053.926	123.663	156.444	1474.680	0.990	28.524
18	3	2151.550	27012.949	5221.319	53358.955	2671.754	4714.460	79.641	78.576	1249.884	0.787	15.994
19	3	2086.912	27414.847	5190.612	57026.853	2784.667	4120.064	104.997	105.210	1239.882	0.741	15.617
20	3	2302.959	26608.232	1178.295	63648.387	2482.698	1388.958	24.870	26.139	338.102	0.230	3.594
22	4	1647.170	21355.159	1288.963	44654.517	1937.367	1611.437	77.952	60.253	444.604	0.231	4.282
24	4	1381.511	20677.147	2008.926	49196.471	2039.390	1660.351	51.672	32.849	701.952	0.297	5.652
27	4	1203.810	19189.758	1336.984	45086.039	2054.224	1131.563	48.754	27.693	423.886	0.179	4.000
29	4	1398.409	22417.259	2369.393	43054.190	2562.379	2216.045	78.804	46.103	618.226	0.355	5.301
31	5	1660.947	22664.753	4794.338	48541.970	2124.676	4297.473	181.257	63.591	1181.327	0.627	11.130
33	5	1386.057	17272.298	1459.336	42172.261	2042.441	1887.060	81.131	37.479	519.850	0.277	5.512
35	5	1415.100	17547.127	503.049	41233.870	1921.267	648.484	21.151	8.822	159.529	0.085	1.385
37	5	2629.654	33774.014	1228.087	58525.952	2298.876	718.841	28.056	11.103	253.651	0.113	1.821
39	5	2591.367	32557.727	5695.158	58554.172	2175.030	2391.704	115.951	47.276	1150.021	0.569	8.932
41	5	1777.380	22910.752	1017.303	42351.955	2332.192	931.371	61.276	26.050	239.391	0.147	2.804

Total metals - Pore-water (µg/L) - SRBR 8

Week	MIW batch	Na	Mg	Al	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Pb
6	2	5465.333	17364.954	130.683	37576.571	524.592	367.404	210.713	17.204	41.689	0.096	0.890
7	2	4494.704	15864.270	82.554	49481.573	941.831	478.634	356.297	10.430	28.010	0.025	0.838

8	2	3072.229	16835.427	120.194	44527.376	1271.821	562.867	55.101	8.521	45.175	0.048	0.765
9	2	3902.626	19480.540	58.186	50966.941	1492.193	433.074	36.199	6.103	24.805	0.015	0.786
10	2	4726.271	24450.141	163.304	58636.215	1586.845	485.507	266.599	17.675	72.560	0.042	0.945
11	2	4175.913	23113.897	226.399	53739.912	1675.518	482.334	367.257	15.528	83.896	0.050	1.268
12	2	4025.066	22699.068	568.219	51864.936	1624.375	581.300	25.306	13.183	127.054	0.096	1.256
13	2	3965.409	21320.452	163.345	53923.023	1542.501	276.314	387.646	10.445	57.948	0.035	1.019
14	3	2939.236	26122.086	314.837	60323.110	2413.704	1387.006	47.717	14.623	85.247	0.043	0.636
15	3	2428.817	25132.681	1578.462	59178.637	2328.075	2721.859	37.480	62.123	411.984	0.282	3.727
16	3	2977.781	32733.577	1688.129	70785.818	2478.140	2751.216	55.506	63.726	360.118	0.241	3.289
17	3	1842.990	19497.505	791.001	50520.291	1844.375	1357.093	372.310	32.135	283.813	0.179	3.404
18	3	2205.997	26122.075	2646.688	56285.211	2227.714	2892.043	62.923	57.474	800.345	0.482	6.120
19	3	2162.366	27250.049	183.845	54765.394	2586.463	1500.350	44.012	11.710	104.630	0.027	0.450
20	3	2244.286	27660.686	630.677	66696.965	2312.702	1151.891	68.569	18.373	205.868	0.106	1.528

Appendix A-2: Summary of 2-way ANOVA statistical analyses performed on the water quality data showing the influence of the hydraulic retention time (HRT, ~ 3 and ~ 10 days) and the alkalinity source (mussel shell vs. limestone).

	HRT	**Alkalinity source**
pH (effluent)	***	***
ORP (pore-water)	***	0.360
Conductivity (effluent)	0.776	**
D.O. (effluent)	**	0.075
Alkalinity (total)	***	***
Alkalinity ($CaCO_3$ contribution)	*	***
Alkalinity (SRB contribution)	***	0.816
Sulfate concentration	**	0.984
Sulfate reduction (%)	***	0.682
Sulfate reduction ($mol/m^3/d$)	0.274	0.432
Sulfide concentration	0.842	***
Nitrate concentration (effluent)	0.770	**
Nitrate concentration (pore-water)	0.144	0.840
Phosphate concentration (effluent)	0.547	***
Phosphate concentration (pore-water)	0.655	0.320
COD concentration (effluent)	***	0.830
COD concentration (pore-water)	*	0.094
TOC concentration (effluent)	*	*
DOC concentration (effluent)	**	0.081
Fe removal (%)	***	***
Al removal (%)	**	**
Mn removal (%)	***	*
Zn removal (%)	**	***
Ni removal (%)	***	***
Cu removal (%)	*	*
Cd removal (%)	*	**

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Appendix A-3: Sequential extraction raw data. 2A = SRBR 2 top; 2B = SRBR 2 bottom; 3A = SRBR 3 top; 3B = SRBR 3 bottom; 5A = SRBR 5 top; 5B = SRBR 5 bottom; 7A = SRBR 7 top; 7B = SRBR 7 bottom; S1 to S6 = sequential extraction steps 1 to 6; W1 to W5 = washes in-between extraction steps.

Sample ID		Al	Mn	Fe	Ni	Cu	Zn	Cd
2A1	S1	1.125	18.434	0.649	3.222	0.652	0.556	0.006
2A2	S1	1.634	17.298	0.576	3.664	0.706	0.757	0.010
2A3	S1	1.261	17.536	0.725	2.439	0.553	0.605	0.012
2A1	W1	0.359	6.959	0.264	1.510	0.217	0.382	0.021
2A2	W1	0.696	2.767	0.185	2.185	0.213	0.231	0.003
2A3	W1	0.660	2.942	0.184	1.937	0.209	0.230	0.003
2A1	S2	0.887	207.708	3.360	1.802	0.423	0.678	0.017
2A2	S2	1.053	208.183	3.372	1.700	0.202	0.687	0.018
2A3	S2	1.365	231.697	3.742	1.886	0.224	0.745	0.016
2A1	W2	1.237	33.438	1.286	2.496	0.257	0.397	0.007
2A2	W2	1.195	30.517	0.867	1.925	0.218	0.443	0.010
2A3	W2	1.150	31.523	0.946	1.854	0.218	0.320	0.008
2A1	S3	0.493	180.299	17.631	1.266	0.158	8.725	0.014
2A2	S3	0.487	175.733	18.580	1.308	0.169	8.037	0.009
2A3	S3	0.479	165.683	17.435	1.316	0.157	7.502	0.008
2A1	W3	0.154	15.846	2.186	0.845	0.081	0.574	0.005
2A2	W3	0.127	15.808	2.021	0.926	0.081	0.530	0.002
2A3	W3	0.121	16.476	2.066	0.838	0.076	0.588	0.002
2A1	S4	288.974	111.097	547.202	3.734	0.169	126.667	0.326
2A2	S4	242.292	100.723	455.509	3.325	0.096	106.609	0.274
2A3	S4	277.262	107.857	504.948	3.626	0.117	116.381	0.288
2A1	W4	19.012	8.336	44.023	1.145	0.067	10.948	0.017
2A2	W4	22.118	10.523	53.716	1.172	0.077	13.299	0.022
2A3	W4	21.809	10.167	55.026	1.398	0.065	12.495	0.021
2A1	S5	809.740	15.793	1094.137	11.289	16.162	36.824	0.124
2A2	S5	872.841	17.041	1198.423	12.420	17.992	43.658	0.144
2A3	S5	825.310	16.436	1168.678	12.028	16.875	41.482	0.134
2A1	W5	107.709	2.604	152.628	2.014	2.616	5.821	0.041
2A2	W5	64.496	1.861	94.566	1.339	1.671	3.823	0.014
2A3	W5	149.897	3.448	218.390	2.774	3.434	8.392	0.032
2A1	S6	7335.677	50.040	3461.371	14.126	14.617	48.175	0.106
2A1	S6	6441.519	45.196	3080.667	13.082	13.057	45.614	0.125
2A2	S6	3438.417	26.831	2149.830	6.036	7.924	15.961	0.055
2A3	S6	5221.766	36.062	2748.768	15.141	7.314	16.532	0.158
2A3	S6	4913.907	34.081	2630.202	14.347	7.089	14.205	0.145

2B1	S1	2.092	15.595	1.838	5.533	0.172	0.261	0.005
2B2	S1	2.020	15.837	1.244	5.365	0.175	0.482	0.008
2B3	S1	2.276	16.686	1.376	2.981	0.173	0.342	0.005
2B1	W1	0.398	2.145	0.230	2.586	0.060	0.105	0.002
2B2	W1	0.452	2.531	0.374	2.002	0.068	0.160	0.014
2B3	W1	0.607	2.508	0.313	2.574	0.066	0.115	0.003
2B1	S2	0.766	120.597	3.572	1.184	0.065	0.201	0.007
2B2	S2	1.088	156.850	4.030	1.695	0.070	0.248	0.009
2B3	S2	1.213	151.501	4.409	1.303	0.076	0.259	0.008
2B1	W2	0.568	15.272	0.807	1.085	0.063	0.102	0.001
2B2	W2	0.842	23.517	1.005	1.244	0.074	0.134	0.001
2B3	W2	0.726	22.222	0.929	1.210	0.069	0.107	0.001
2B1	S3	0.426	159.593	14.981	0.770	0.070	2.281	0.006
2B2	S3	0.452	202.305	18.554	0.624	0.104	2.716	0.008
2B3	S3	0.450	183.003	17.740	0.583	0.107	2.384	0.006
2B1	W3	0.198	18.536	2.162	0.679	0.042	0.212	0.001
2B2	W3	0.166	20.706	2.254	0.646	0.044	0.256	0.001
2B3	W3	0.180	15.214	1.963	0.642	0.039	0.219	0.001
2B1	S4	267.289	121.267	670.022	3.396	0.238	107.930	0.334
2B2	S4	230.635	146.570	658.812	3.061	0.237	98.781	0.303
2B3	S4	261.145	128.570	665.486	2.922	0.226	107.406	0.314
2B1	W4	16.326	6.697	44.724	0.774	0.076	7.425	0.010
2B2	W4	18.355	11.291	58.501	0.844	0.084	8.741	0.012
2B3	W4	20.836	9.331	59.722	0.940	0.090	9.419	0.012
2B1	S5	1111.115	18.355	1354.529	10.430	14.012	40.291	0.121
2B2	S5	1310.064	47.504	1856.059	11.772	16.402	49.894	0.151
2B3	S5	1329.151	22.244	1651.300	12.382	16.039	49.425	0.144
2B1	W5	88.259	2.007	113.989	1.217	1.483	3.794	0.021
2B2	W5	81.492	3.296	117.491	1.274	1.409	3.732	0.017
2B3	W5	94.725	1.889	126.909	1.660	1.515	3.992	0.014
2B1	S6	7331.065	51.927	2777.864	12.854	10.833	31.218	0.198
2B1	S6	7380.165	53.648	2809.918	13.015	11.050	31.696	0.209
2B2	S6	5443.511	36.554	2669.740	6.421	34.508	15.992	0.028
2B3	S6	7086.918	38.424	2908.551	7.708	10.467	31.180	0.079
2B3	S6	6693.925	36.596	2768.978	7.309	10.133	30.906	0.080
3A1	S1	0.653	17.011	0.846	0.151	0.156	0.341	0.000
3A2	S1	0.822	16.119	0.900	0.138	0.121	0.535	0.006
3A3	S1	0.631	16.065	0.846	0.132	0.117	0.324	0.000
3A1	W1	0.415	3.505	0.258	0.052	0.068	0.290	0.000

3A2	W1	0.401	3.560	0.280	0.072	0.072	0.307	0.000
3A3	W1	0.359	2.995	0.226	0.051	0.061	0.199	0.025
3A1	S2	0.409	91.055	3.072	0.470	0.051	1.460	0.027
3A2	S2	0.555	106.021	3.554	0.535	0.060	1.667	0.028
3A3	S2	0.570	100.921	3.852	0.515	0.054	1.536	0.158
3A1	W2	0.562	12.987	0.930	0.129	0.126	0.501	0.002
3A2	W2	0.485	13.957	0.843	0.129	0.047	0.312	0.002
3A3	W2	0.571	14.388	0.912	0.145	0.056	0.368	0.004
3A1	S3	0.419	158.817	17.946	1.167	0.116	22.036	0.028
3A2	S3	0.437	155.651	17.531	1.135	0.187	20.517	0.022
3A3	S3	0.454	154.653	17.797	1.163	0.218	20.632	0.022
3A1	W3	0.115	18.516	2.644	0.214	0.034	1.366	0.003
3A2	W3	0.128	17.935	2.598	0.278	0.050	1.310	0.001
3A3	W3	0.115	17.054	2.594	0.225	0.031	1.296	0.001
3A1	S4	302.781	89.962	703.280	6.865	0.186	144.512	0.309
3A2	S4	291.661	88.019	680.202	6.770	0.164	138.248	0.300
3A3	S4	295.866	92.169	688.290	6.644	0.162	139.676	0.301
3A1	W4	18.838	6.033	49.733	0.842	0.069	12.315	0.017
3A2	W4	20.874	6.709	54.375	1.087	0.074	13.281	0.017
3A3	W4	19.718	6.570	50.723	1.033	0.079	12.431	0.015
3A1	S5	804.268	15.225	1169.270	22.810	14.446	27.558	0.080
3A2	S5	780.825	14.896	1152.133	22.385	13.296	26.715	0.077
3A3	S5	688.292	13.894	1029.618	20.683	12.009	23.714	0.067
3A1	W5	57.085	1.275	82.526	1.733	1.326	2.634	0.007
3A2	W5	69.804	1.483	103.693	2.189	1.459	3.001	0.005
3A3	W5	54.072	1.212	78.746	1.703	1.152	2.557	0.004
3A1	S6	2447.538	13.935	1133.277	6.228	6.424	14.170	0.016
3A2	S6	4048.592	18.867	1532.763	10.179	7.026	14.621	0.010
3A3	S6	3123.247	16.932	1350.589	15.206	5.598	11.348	0.071
3B1	S1	0.837	18.309	1.162	0.092	0.960	0.492	0.000
3B2	S1	0.856	17.782	1.097	0.095	0.949	0.392	0.000
3B3	S1	0.941	20.552	1.141	0.135	1.080	0.494	0.000
3B1	W1	0.383	3.176	0.361	0.034	0.381	0.227	0.000
3B2	W1	0.407	2.716	0.259	0.024	0.317	0.191	0.000
3B3	W1	0.335	3.121	0.267	0.029	0.362	0.188	0.000
3B1	S2	0.819	156.123	5.840	0.391	0.150	2.512	0.069
3B2	S2	0.973	169.118	5.827	0.412	0.082	2.554	0.066
3B3	S2	0.969	173.534	5.696	0.411	0.087	2.557	0.069
3B1	W2	0.593	17.119	0.865	0.084	0.061	0.362	0.000

3B2	W2	0.742	18.395	0.908	0.098	0.066	0.450	0.001
3B3	W2	0.917	18.309	0.977	0.101	0.080	0.396	0.000
3B1	S3	0.499	163.102	24.219	0.655	0.186	22.509	0.049
3B2	S3	0.495	151.645	23.367	0.625	0.236	20.134	0.040
3B3	S3	0.518	163.402	26.515	0.675	0.318	22.658	0.048
3B1	W3	0.122	14.486	2.893	0.142	0.037	1.059	0.000
3B2	W3	0.197	14.128	2.670	0.207	0.066	1.013	0.002
3B3	W3	0.185	13.901	2.825	0.240	0.070	1.043	0.000
3B1	S4	288.854	103.188	692.863	4.480	0.258	154.817	0.321
3B2	S4	248.137	90.991	616.441	4.035	0.244	138.191	0.289
3B3	S4	315.851	120.600	801.077	5.279	0.286	153.558	0.351
3B1	W4	18.114	7.332	53.796	0.710	0.104	15.287	0.016
3B2	W4	18.562	7.226	47.902	0.632	0.095	13.616	0.013
3B3	W4	16.329	6.932	49.251	0.585	0.089	11.699	0.014
3B1	S5	1129.521	17.241	1595.733	18.975	19.471	39.215	0.104
3B2	S5	1019.535	17.642	1406.425	16.756	16.959	35.426	0.094
3B3	S5	1180.557	18.486	1619.398	20.475	18.341	36.118	0.094
3B1	W5	62.709	1.026	92.210	1.172	1.389	2.828	0.005
3B2	W5	65.921	1.178	91.115	1.141	1.344	2.865	0.007
3B3	W5	59.805	1.017	86.350	1.138	1.276	6.997	0.003
3B1	S6	4070.294	23.296	1968.619	4.046	7.381	17.213	0.059
3B2	S6	5213.577	26.019	2207.888	3.404	7.755	18.688	0.028
3B3	S6	7896.647	39.753	3304.639	6.427	10.923	26.744	0.000
5A1	S1	1.862	14.923	1.118	0.702	1.939	10.674	0.000
5A2	S1	1.049	14.019	0.896	0.569	1.417	7.712	0.000
5A3	S1	1.482	14.445	1.170	0.616	1.263	6.379	0.000
5A1	W1	0.516	1.627	0.238	0.382	0.394	2.057	0.000
5A2	W1	0.446	1.692	0.183	0.573	0.484	2.042	0.018
5A3	W1	0.400	1.538	0.167	0.360	0.353	2.136	0.001
5A1	S2	0.711	154.323	2.111	0.899	0.386	3.019	0.056
5A2	S2	0.951	132.652	2.429	0.768	0.353	2.716	0.050
5A3	S2	0.890	172.170	2.742	1.036	0.297	3.045	0.078
5A1	W2	0.782	21.757	0.563	0.336	0.288	1.376	0.001
5A2	W2	0.697	20.559	0.526	0.297	0.212	1.028	0.000
5A3	W2	0.867	20.925	0.578	0.318	0.212	0.912	0.000
5A1	S3	0.756	174.135	15.500	0.726	0.171	18.010	0.051
5A2	S3	1.084	168.487	15.430	0.737	0.148	16.987	0.047
5A3	S3	0.925	174.005	16.018	0.756	0.156	17.837	0.056
5A1	W3	0.196	14.344	1.810	0.074	0.081	0.964	0.000

5A2	W3	0.191	13.161	1.755	0.069	0.082	1.367	0.000
5A3	W3	0.386	13.385	1.825	0.079	0.093	1.031	0.000
5A1	S4	323.523	206.105	758.169	6.274	0.717	114.475	0.433
5A2	S4	343.028	234.288	839.783	6.669	0.640	119.735	0.468
5A3	S4	341.179	211.663	794.116	6.564	0.662	117.521	0.437
5A1	W4	24.605	14.694	64.186	0.495	0.329	9.079	0.013
5A2	W4	24.320	15.223	60.366	0.476	0.322	8.552	0.014
5A3	W4	21.794	12.954	61.250	0.469	0.246	8.005	0.011
5A1	S5	1643.429	33.606	2376.155	29.205	18.959	34.668	0.123
5A2	S5	1665.925	32.529	2478.627	29.891	20.801	34.342	0.138
5A3	S5	1644.101	32.235	2381.796	29.419	18.941	37.120	0.132
5A1	W5	74.497	1.575	124.712	1.364	1.662	3.014	0.008
5A2	W5	68.661	1.442	117.794	1.287	1.858	2.701	0.019
5A3	W5	62.134	1.371	102.422	1.167	1.452	2.379	0.010
5A1	S6	24062.528	121.016	17972.658	96.275	73.394	308.175	0.153
5A2	S6	22600.327	91.564	17919.685	41.548	56.296	152.637	0.075
5A3	S6	27441.238	118.237	22037.870	50.106	66.516	190.992	0.083
5B1	S1	3.420	11.896	2.566	0.926	3.195	3.046	0.002
5B2	S1	4.260	11.886	1.917	0.628	3.214	3.667	0.001
5B3	S1	4.469	11.533	2.249	0.968	3.281	3.128	0.001
5B1	W1	1.610	1.383	0.384	0.479	1.087	1.008	0.001
5B2	W1	2.467	1.462	0.660	0.440	1.087	1.087	0.001
5B3	W1	1.569	1.333	0.339	0.384	1.089	1.306	0.001
5B1	S2	2.036	117.418	2.899	0.965	1.141	2.578	0.024
5B2	S2	1.882	102.805	2.580	0.819	1.026	2.218	0.021
5B3	S2	1.952	103.895	2.709	0.816	1.052	2.287	0.021
5B1	W2	1.731	20.192	0.694	0.346	1.152	1.299	0.000
5B2	W2	1.930	18.433	0.679	0.457	1.145	1.444	0.001
5B3	W2	1.934	18.355	0.666	0.327	1.152	1.293	0.002
5B1	S3	1.030	87.091	12.823	0.698	0.489	18.727	0.022
5B2	S3	1.100	86.821	13.552	0.710	0.484	19.356	0.027
5B3	S3	1.418	93.102	14.474	0.762	0.503	20.275	0.026
5B1	W3	0.254	10.229	2.233	0.100	0.429	2.367	0.000
5B2	W3	0.335	9.841	2.231	0.106	0.742	2.422	0.003
5B3	W3	0.244	9.694	2.094	0.091	0.381	2.316	0.001
5B1	S4	451.496	86.942	758.395	6.331	0.923	166.282	0.380
5B2	S4	443.039	85.163	722.698	5.859	0.720	149.294	0.390
5B3	S4	471.447	94.706	767.687	6.059	0.830	157.203	0.413
5B1	W4	40.528	7.088	73.139	0.558	0.377	15.971	0.017

5B2	W4	41.489	7.291	72.484	0.583	0.389	15.954	0.018
5B3	W4	38.286	7.288	66.318	0.570	0.399	15.400	0.017
5B1	S5	1672.821	20.934	1785.664	24.618	25.654	76.444	0.157
5B2	S5	1638.549	20.309	1779.347	25.509	25.359	71.377	0.160
5B3	S5	1618.826	21.457	1838.255	26.130	25.270	71.647	0.166
5B1	W5	104.234	1.438	126.220	1.692	2.889	5.547	0.022
5B2	W5	94.616	1.368	122.365	1.562	3.599	6.075	0.040
5B3	W5	96.380	1.347	122.583	1.625	2.697	5.529	0.022
5B1	S6	36248.838	190.150	20222.836	91.118	73.577	171.671	0.484
5B2	S6	26576.380	165.428	18068.512	99.780	59.194	148.114	0.378
5B3	S6	42645.337	214.507	25291.951	98.948	81.657	194.316	0.514
7A1	S1	4.644	12.650	0.929	0.080	0.887	1.849	0.006
7A2	S1	2.308	12.266	0.906	0.073	1.081	2.006	0.007
7A3	S1	3.617	12.407	1.179	0.077	0.841	1.567	0.013
7A1	W1	0.689	1.339	0.361	0.040	0.389	0.708	0.002
7A2	W1	0.942	1.366	0.391	0.064	0.466	1.137	0.002
7A3	W1	0.916	1.359	0.657	0.050	0.389	0.892	0.002
7A1	S2	0.239	77.540	2.061	0.112	0.268	0.889	0.063
7A2	S2	0.253	73.994	1.953	0.110	0.262	0.878	0.059
7A3	S2	0.285	73.460	1.995	0.115	0.337	1.014	0.066
7A1	W2	0.165	9.563	0.468	0.035	0.400	0.600	0.003
7A2	W2	0.206	9.769	0.458	0.033	0.395	0.583	0.002
7A3	W2	0.241	9.579	0.413	0.032	0.404	0.657	0.004
7A1	S3	0.389	91.884	11.220	0.207	0.155	5.781	0.052
7A2	S3	0.424	97.588	11.783	0.258	0.134	5.900	0.051
7A3	S3	0.423	95.423	11.951	0.206	0.125	5.847	0.053
7A1	W3	0.104	10.349	2.175	3.643	0.077	0.428	0.007
7A2	W3	0.044	9.900	2.007	0.092	0.071	0.366	0.003
7A3	W3	0.069	9.659	1.958	0.093	0.068	0.428	0.006
7A1	S4	338.611	118.013	871.328	2.118	0.549	67.073	0.520
7A2	S4	339.613	121.385	878.869	2.167	0.477	66.465	0.530
7A3	S4	320.427	112.282	844.569	2.112	0.533	65.207	0.507
7A1	W4	18.090	6.385	58.045	0.155	0.209	5.042	0.021
7A2	W4	18.794	6.843	57.640	0.166	0.205	4.967	0.022
7A3	W4	17.901	6.385	55.182	0.154	0.175	4.926	0.020
7A1	S5	1660.533	23.855	2170.208	13.168	11.452	17.961	0.116
7A2	S5	1630.518	24.594	2253.242	13.743	11.749	18.003	0.122
7A3	S5	1616.022	23.619	2181.305	13.172	11.516	18.854	0.124
7A1	W5	126.129	2.011	175.944	1.224	1.463	4.939	0.033

7A2	W5	131.800	2.161	189.026	1.354	1.714	5.156	0.018
7A3	W5	125.423	2.019	183.328	1.431	2.115	4.957	0.018
7A1	S6	18517.750	92.652	13548.635	42.751	32.153	74.643	0.116
7A2	S6	16014.369	76.303	11602.795	32.105	27.741	71.717	0.133
7A3	S6	17184.055	82.476	12413.780	31.067	28.653	124.991	0.117
7B1	S1	4.114	9.007	1.216	0.139	2.587	2.759	0.004
7B2	S1	3.953	8.642	1.114	0.102	2.493	1.932	0.005
7B3	S1	4.017	8.752	1.154	0.104	2.466	1.876	0.005
7B1	W1	0.242	1.362	0.282	0.033	1.044	0.682	0.001
7B2	W1	1.022	1.027	0.375	0.031	0.944	0.944	0.002
7B3	W1	0.952	1.018	0.427	0.041	0.942	0.781	0.002
7B1	S2	0.299	82.207	2.234	0.148	0.862	0.804	0.018
7B2	S2	0.295	78.789	2.380	0.134	0.795	0.839	0.016
7B3	S2	0.274	74.359	1.876	0.138	0.746	0.755	0.015
7B1	W2	0.232	10.402	0.481	0.045	0.912	0.655	0.002
7B2	W2	0.277	11.314	0.608	0.056	0.872	0.705	0.003
7B3	W2	0.193	8.673	0.505	0.034	0.666	0.579	0.001
7B1	S3	0.563	74.306	11.262	0.219	0.149	2.953	0.013
7B2	S3	0.508	72.133	10.277	0.217	0.138	3.066	0.015
7B3	S3	0.536	77.255	11.709	0.233	0.127	2.899	0.013
7B1	W3	0.121	7.287	1.884	0.162	0.116	0.262	0.025
7B2	W3	0.111	7.886	1.839	0.150	0.104	0.298	0.002
7B3	W3	0.092	8.010	1.798	0.112	0.099	0.258	0.002
7B1	S4	375.385	138.000	960.105	3.392	0.797	84.234	0.573
7B2	S4	336.908	137.406	862.739	2.947	0.791	77.827	0.510
7B3	S4	326.243	130.881	838.050	2.960	0.758	77.826	0.534
7B1	W4	17.855	6.651	61.037	0.217	0.306	5.515	0.017
7B2	W4	17.826	7.519	58.099	0.213	0.268	5.889	0.018
7B3	W4	18.002	7.203	54.745	0.212	0.275	5.576	0.017
7B1	S5	1766.658	27.944	2298.963	19.473	13.195	34.759	0.186
7B2	S5	1786.876	27.951	2287.544	19.409	12.853	35.455	0.189
7B3	S5	1679.587	26.994	2216.627	18.435	12.672	34.270	0.178
7B1	W5	118.244	2.174	174.923	1.595	1.603	6.878	0.023
7B2	W5	133.472	2.342	195.268	1.756	1.673	6.957	0.024
7B3	W5	130.263	2.271	186.005	1.669	1.547	6.599	0.022
7B1	S6	19039.295	108.311	12102.175	24.560	27.469	646.035	0.208
7B2	S6	22493.174	116.428	13604.988	44.334	35.052	103.054	0.200
7B3	S6	23361.353	122.421	14337.887	50.119	35.045	101.759	0.169
Water Blank	S1	0.365	0.019	0.076	0.532	0.116	0.137	0.014

Water Blank	W1	0.128	0.013	0.071	0.244	0.039	0.096	0.006
Water Blank	S2	0.200	0.592	0.333	0.347	0.034	0.109	0.747
Water Blank	W2	0.106	0.019	0.181	0.363	0.063	0.109	0.006
Water Blank	S3	0.152	0.198	0.774	0.326	0.088	0.099	0.009
Water Blank	W3	0.099	0.007	0.204	0.214	0.033	0.187	0.002
Water Blank	S4	2.372	0.394	4.347	0.820	0.203	1.321	0.021
Water Blank	W4	0.142	0.015	0.195	0.079	0.058	0.330	0.002
Water Blank	S5	1.877	0.080	0.713	0.395	0.257	1.159	0.033
Water Blank	W5	0.072	0.011	0.200	0.095	0.044	0.333	0.003
Water Blank	S6	10.321	0.087	4.283	0.487	1.814	2.199	0.098

Appendix A-4: Gas analyses.

Author(s):	**Ben Rumsey**
CRL Ref:	**14-11009**
Title:	**Gas Analysis Report**
Client Name:	**Ben Uster** **Canterbury University**
Client Address:	
Date of Issue:	**4 March 2014**
Signature: *Name & Designation:*	 **Ben Rumsey** **Research Officer**
Distribution: *(other than client)*	CRL Energy Laboratory

CRL Energy Ltd

88 Gracefield Road,
PO Box 31-244
Lower Hutt
New Zealand
TEL +64 4 570 3700
FAX +64 4 570 3701
www.crl.co.nz

CHRISTCHURCH OFFICE
123B Blenheim Road
PO Box 29-415
Christchurch
New Zealand
TEL +64 3 341 2120
FAX +64 3 341 5600

HAMILTON OFFICE
C/- Ruakura Research Centre
Private Bag 3123
Hamilton
New Zealand
TEL +64 7 838 5281
FAX +64 7 838 5282

WEST COAST LABORATORY
43 Arney Street
PO Box 290
Greymouth
New Zealand
TEL +64 3 768 0586
FAX +64 3 768 0587

Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7

CH_4	0.0030	0.0098	0.0169	0.0039	0.0071	0.0090	0.0099
CO_2	3.07	3.91	5.29	2.60	4.52	5.89	5.91
C_2H_4	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
C_2H_6	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
H_2	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
O_2	19.63	15.50	18.44	20.03	13.66	14.59	17.59
N_2	74.12	80.02	75.35	76.58	81.63	78.84	75.50
CO	< 0.002	< 0.002	< 0.002	< 0.002	0.0112	< 0.002	< 0.002
H_2S	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Values are expressed on a mole/mole % basis. The tests were conducted with an Agilent 3000A TCD micro gas chromatograph, using CRL Energy Ltd's in-house procedures and BOC Alpha and Beta calibration gasses. H_2S tested with a Crowcom Tetra 3 with no detectable H_2S present.

Appendix A-5: Initial and final pH measured during the adsorption edge experiment.

	Initial pH												
Mn	3.02	3.51	3.99	4.49	5.00	5.51	5.98	6.48	7.01	7.51	8.01	8.51	9.01
Zn	3.02	3.51	4.02	4.51	5.01	5.48	6.01	6.51	7.02	7.52	7.98	8.52	8.99
Mn-control[a]	3.02	3.51	4.03	4.54	5.04	5.49	6.00	6.49	6.98	7.49	7.97	8.51	8.95
Zn-control[a]	3.01	3.49	4.02	4.49	5.02	5.51	5.97	6.50	6.97	7.51	7.97	8.48	9.01
Control#2[b]	3.02	3.5	3.98	4.48	4.97	5.49	6.01	6.49	6.97	7.48	7.99	8.51	8.99
	Final pH (after 24 h agitation)												
Mn	2.98	3.28	3.82	4.15	4.62	5.06	5.54	5.99	6.31	6.73	6.95	7.41	7.79
Zn	3.08	3.82	4.19	4.36	4.74	5.10	5.49	5.85	6.22	6.54	6.92	7.36	7.71
Mn-control[a]	3.12	3.57	4.07	4.55	4.95	5.27	5.55	5.86	6.15	6.32	6.48	6.60	7.10
Zn-control[a]	2.97	3.46	3.96	4.52	4.79	5.12	5.43	5.62	6.05	6.30	6.58	6.78	7.94
Control#2[b]	3.02	3.44	3.85	4.27	4.66	4.98	5.28	5.62	6.02	6.45	6.81	7.12	7.50

[a] = metal solution without solids,
[b] = organic material without metal.

Appendix A-6: PHREEQC input parameters.

Parameters	Unit	MS-S	MS-L	LS-S	LS-L
pH	-	6.43	6.72	6.14	6.36
ORP	mV	- 70	- 216	- 115	- 199
Fe	mg/L	27.4	27.4	27.4	27.4
Al	mg/L	15.5	15.5	15.5	15.5
Mn	mg/L	15.8	15.8	15.8	15.8
Zn	mg/L	4.8	4.8	4.8	4.8
Cu	mg/L	0.23	0.23	0.23	0.23
Ni	mg/L	1	1	1	1
Sulfate	mg/L	1708	1708	1708	1708
NO_3^-		1.5	1.5	1	1.2
PO_4^{3-}		5	5.75	1	1.5
Ca		145.2	324.6	161.1	270.6
Mg		138.9	129.5	140.5	129.3
Na		14.9	15.7	13.0	13.8

Appendix A-7: SI values computed using PHREEQC model.

	Mineral phases	Formula	MS-S	MS-L	LS-S	LS-L
Fe	Ferrihydrite	$Fe(OH)_3$	-2.67	-9.66	-3.89	-8.30
	Goethite	α-FeO(OH)	0.10	-6.89	-1.13	-5.54
	Vivianite	$Fe_3(PO_4)_2{\cdot}H_2O$	2.42	-8.04	0.03	-8.46
	Siderite	$FeCO_3$	-0.70	-3.76	-1.08	-3.70
	Mackinawite	FeS	-10.22	0.23	-5.01	0.22
	Greigite	Fe_3S_4	-35.25	2.22	-15.15	5.19
	Pyrite	FeS_2	-7.39	9.19	2.30	12.16
Al	Gibbsite	$Al(OH)_3$	4.18	4.27	3.92	4.16
	Al hydroxide	$Al(OH)_3$(am)	1.53	1.68	1.33	1.58
	Boehmite	AlOOH	3.78	3.87	3.52	3.77
	Basaluminite	$Al_4(OH)_{10}SO_4$	14.05	-2.03	13.62	9.20
	Al oxide	Al_2O_3	4.94	5.13	4.42	4.92
Mn	Rhodochrosite	$MnCO_3$	-0.46	0.46	-0.85	-0.07
	Mn phosphate	$MnHPO_4$	3.97	4.33	3.05	3.59
	Pyrolusite	MnO_2	-23.57	-30.36	-25.47	-28.13
	Manganite	MnO(OH)	-11.38	-14.38	-12.61	-13.62
Zn	Smithsonite	$ZnCO_3$	-1.71	-9.74	-2.10	-9.48
	Zincite	ZnO	-3.63	-11.81	-4.21	-11.71
	Sphalerite	ZnS	-2.99	2.49	2.21	2.68
	Zn sulfide	ZnS (am)	-5.46	0.02	-0.26	0.21
Ni	Ni carbonate	$NiCO_3$	-5.57	-4.67	-5.96	-5.19
	Ni hydroxide	$Ni(OH)_2$	-5.72	-4.98	-6.30	-5.65
	Bunsenite	NiO	-5.40	-4.65	-5.98	-5.32
	Ni phosphate	$Ni_3(PO_4)_2$	-6.41	-4.98	-8.81	-7.11
	Ni sulfide	NiS(α)	-9.60	4.82	-4.39	4.23
	Ni sulfide	NiS(β)	-4.10	10.32	1.11	9.73
	Ni sulfide	NiS (γ)	-2.40	12.02	2.81	11.43
Cu	Cuprite	CuO_2	2.57	-21.70	1.99	-21.42
	Malachite	$Cu_2(OH)_2CO_3$	-7.20	-38.85	-8.88	-35.53
	Cu carbonate	$CuCO_3$	-4.83	-20.59	-5.59	-18.85
	Covellite	CuS	3.49	1.25	8.33	2.92
	Chalcopyrite	$CuFeS_2$	2.89	11.12	12.95	12.77
	Chalcocite	Cu_2S	14.80	4.21	20.01	4.56

Appendix A-8: Waste acceptance screening criteria for Class A and Class B landfills and concentrations in leachate following the TCLP. Solid screening contents are in mg/kg of dry material, concentrations in leachates are in mg/L (MfE 2004).

	Class A Landfills		Class B Landfills	
Inorganic contaminants	**Solid screening thresholds** [a]	**Concentration in leachates**	**Solid screening thresholds** [a]	**Concentration in leachates**
Aluminium	800	40	80	4
Antimony	12	0.6	1.2	0.06
Arsenic	100	5	10	0.5
Barium	2,000	100	200	10
Beryllium	200	10	20	1
Boron	400	20	40	2
Cadmium	20	1	2	0.1
Chromium (VI)	100	5	10	0.5
Copper	100	5	10	0.5
Fluoride	4,000	200	400	20
Lead	100	5	10	0.5
Lithium	400	20	40	2
Mercury	4	0.2	0.4	0.02
Molybdenum	200	10	20	1
Nickel	200	10	20	1
Selenium	200	1	20	0.11
Silver	200	5	20	0.5
Tin	20,000	1,000	2,000	100
Vanadium	40	2	4	0.2
Zinc	200	10	20	1

[a] = in addition, solid waste content must be at least 20%and liberate no free liquids when transported.

7.2 Appendix B: Supplementary pictures

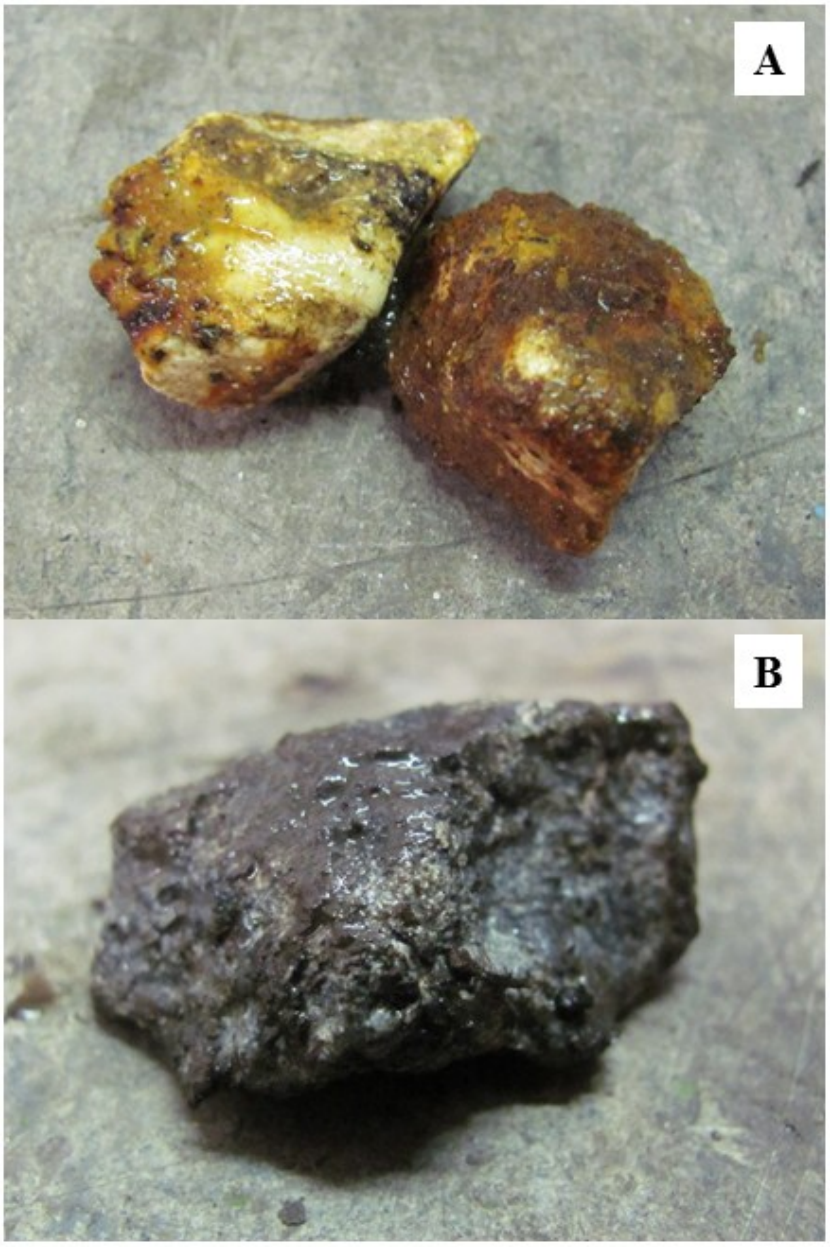


Figure 7-1. Limestone retrieved from: (A) the gravel layer (these two limestone pieces were accidently mixed with the gravels during reactor set-up and were found covered with Fe-(oxy)hydroxides), (B) the spent substrate (no Fe coating observable).

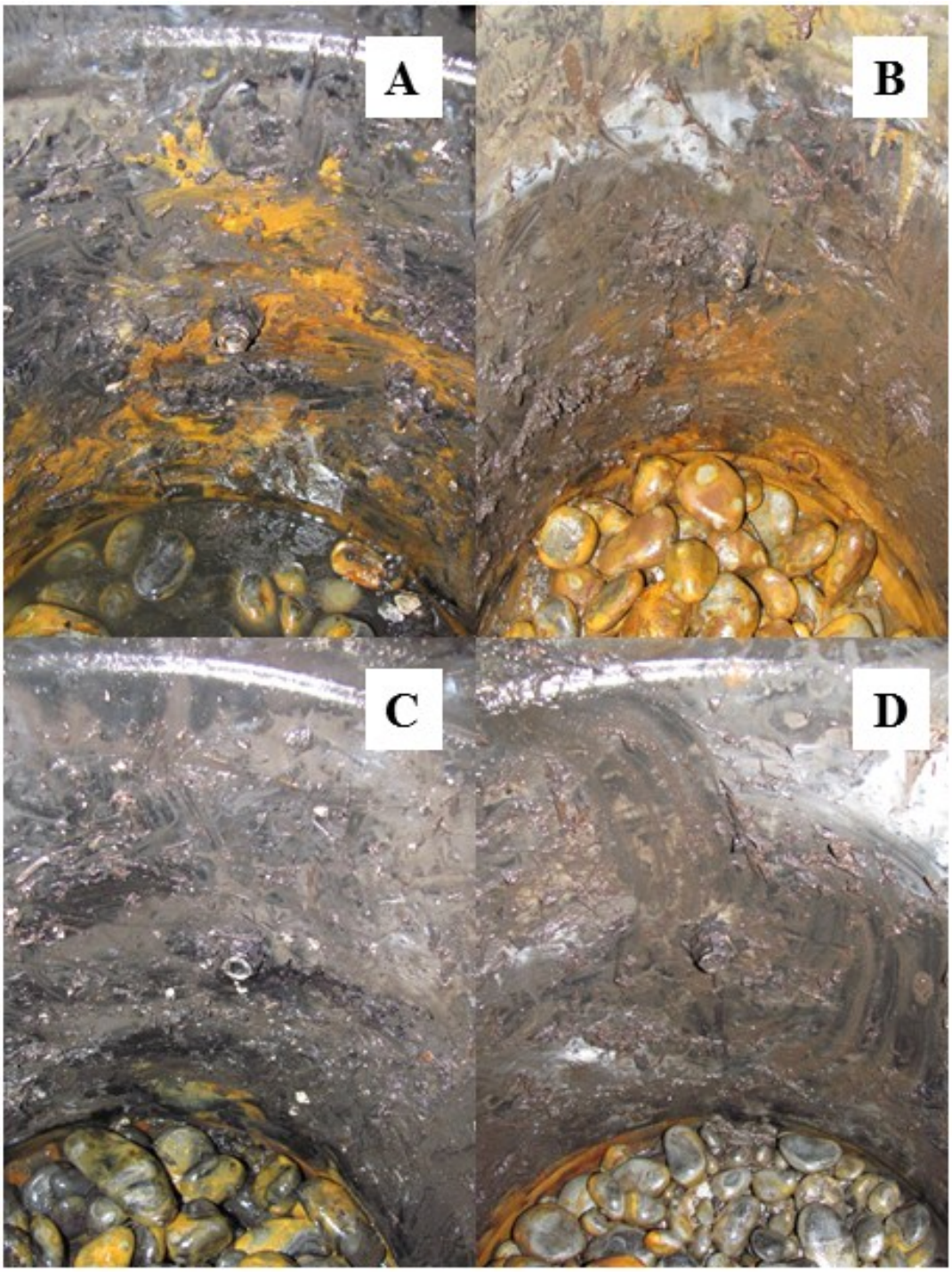


Figure 7-2. Empty reactors post-treatment: (A) mussel shell short HRT, (B) limestone short HRT, (C) mussel shell long HRT, (D) limestone long HRT. The pore-water sampling ports can be seen approximately in the middle of each picture, and reactors MS-S (A) and LS-S (B) show the presence of iron (oxy)hydroxides (i.e. iron ochres).

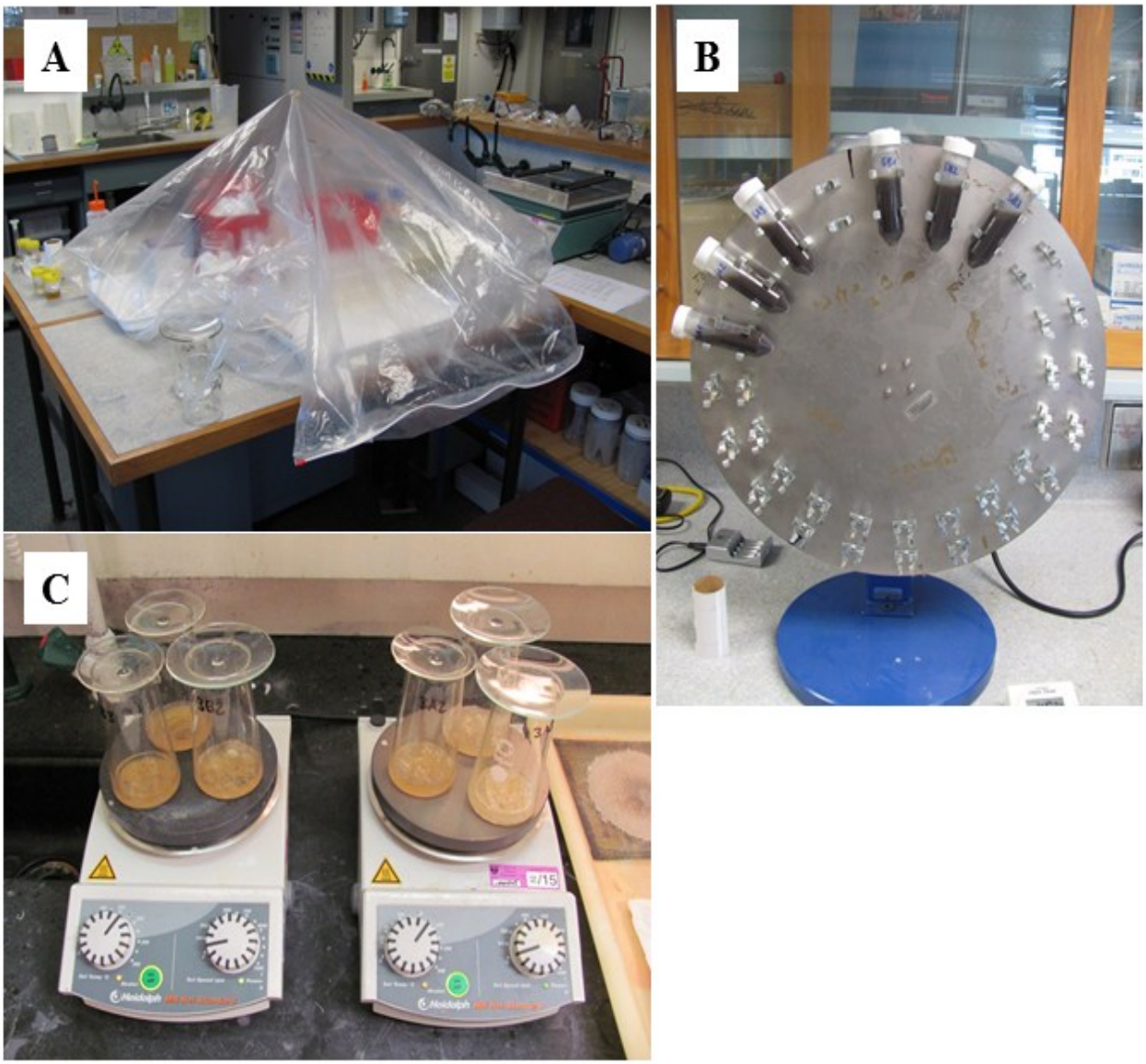


Figure 7-3. Sequential extraction set-up: (A) nitrogen-filled glove-bag, (B) rotary shaker, (C) residual step extraction.

REFERENCES

Abdulkarim A, Tijani IM, Abdulsalam S, Abubakar Jaju M, Alewo OA (2013) Extraction and Characterisation of Chitin and Chitosan from Mussel Shell. Civil and Environmental Research 3 (2):108-114.

Abollino O, Giacomino A, Malandrino M, Mentasti E, Aceto M, Barberis R (2006) Assessment of Metal Availability in a Contaminated Soil by Sequential Extraction. Water Air Soil Pollut 173 (1-4):315-338.

Adams F, Rawajfih Z (1977) Basaluminite and Alunite: A Possible Cause of Sulfate Retention by Acid Soils. Soil Sci Soc Am J 41 (4):686-692.

Amos PW, Younger PL (2003) Substrate characterisation for a subsurface reactive barrier to treat colliery spoil leachate. Water Res 37 (1):108-120.

ANZECC (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality Autralian and New Zealand Environment and Conservation Council (ANZECC),

APHA (2005) Standard Methods for the Examination of Water and Wasterwater. 21st edn. American Public Health Association, Washington D.C.

Appelo CAJ, Parkhurst DL (1999) User Guide to PHREEQC (V.2) A computer program for speciation, batch-reaction, one-dimentional transport, and inverse geochemical calculations. Denver, CO, http://wwwbrr.cr.usgs.gov/projects/GWC_coupled/phreeqc.

Azabou S, Mechichi T, Sayadi S (2007) Zinc precipitation by heavy-metal tolerant sulfate-reducing bacteria enriched on phosphogypsum as a sulfate source. Min Eng 20 (2):173-178.

Bamforth SM, Manning DAC, Singleton I, Younger PL, Johnson KL (2006) Manganese removal from mine waters – investigating the occurrence and importance of manganese carbonates. Appl Geochem 21 (8):1274-1287.

Béchard G, Yamazaki H, Gould WD, Bédard P (1994) Use of Cellulosic Substrates for the Microbial Treatment of Acid Mine Drainage. Journal of Environmental Quality 23 (1):111-116.

Benner SG, Blowes DW, Ptacek CJ, Mayer KU (2002) Rates of sulfate reduction and metal sulfide precipitation in a permeable reactive barrier. Appl Geochem 17 (3):301-320.

Bibby RL, Webster-Brown JG (2006) Trace metal adsorption onto urban stream suspended particulate matter (Auckland region, New Zealand). Appl Geochem 21 (7):1135-1151.

Bigham JM, Nordstrom DK (2000) Iron and Aluminum Hydroxysulfates from Acid Sulfate Waters. Reviews in Mineralogy and Geochemistry 40 (1):351-403.

Bigham JM, Schwertmann U, Traina SJ, Winland RL, Wolff M (1996) Schwertmannite and the chemical modeling of iron in acid sulfate waters. Geochim Cosmochim Acta 60 (12):2111-2121.

Blowes DW, Ptacek CJ, Jambor JL, Weisener CG (2003) The Geochemistry of Acid Mine Drainage. In: Lollar BS (ed) Environmental Geochemistry: Treatise on Geochemistry 9. Elsevier, pp 149-204

Bolis JL, Wildeman TR, Dawson HE (1992) Hydraulic conductivity of substrates used for passive acid mine drainage treatment. In Proc: National Meeting of the American Society for Surface Mining and Reclamation, Duluth, MN, 14-18 June 1992. pp 79-89.

Bosman C (2009) The Hidden Dragon: Nitrate pollution from open-pit mines - a case study from the Limpopo province, South Africa. In Proc: International Mine Water Conference, Pretoria, South Africa, 19-23 October 2009. pp 849-857.

Brown M, Barley B, Wood H (2002) Minewater treatment: Technology, Application and Policy. IWA Publishing, London

Brunet J-F (2000) Drainages miniers acides: Contraintes, remèdes et état des connaissances. Bureau de Recherches Géologiques et Minières (BRGM).

Campbell RN, Lindsay P, Clemens AH (2001) Acid generating potential of waste rock and coal ash in New Zealand coal mines. International Journal of Coal Geology 45 (2–3):163-179.

Carliell-Marquet CM, Wheatley AD (2002) Measuring metal and phosphorus speciation in P-rich anaerobic digesters. Water Sci Technol 45 (10):305-312.

Carroll JJ, Mather AE (1989) The solubility of hydrogen sulphide in water from 0 to 90°C and pressures to 1 MPa. Geochim Cosmochim Acta 53:1163-1170.

Cavanagh J, Pope J, Harding J, Trumm DA, Craw D, Rait R, Greig R, Niyogi D, Buxton R, Champeau O, Clemens A (2010) Framework for predicting and managing water quality impacts of mining on streams: A user's guide. CRL Energy.

Chang IS, Shin PK, Kim BH (2000) Biological treatment of acid mine drainage under sulphate-reducing conditions with solid waste materials as substrate. Water Res 34 (4):1269-1277.

Choudhary RP, Sheoran AS (2011) Comparative study of cellulose waste versus organic waste as substrate in a sulfate reducing bioreactor. Bioresour Technol 102 (6):4319-4324.

Cocos IA, Zagury GJ, Clément B, Samson R (2002) Multiple factor design for reactive mixture selection for use in reactive walls in mine drainage treatment. Water Res 36 (1):167-177.

CoreTeam R (2013) R: A Language and Environment for Statistical Computing. Vienna, Austria, http://www.R-project.org/.

Cornell RM, Schwertmann U (2004) Introduction to the Iron Oxides. In: The Iron Oxides. Chapter 1. Wiley-VCH, pp 1-7. doi:10.1002/3527602097.ch1

Crombie FM, Weber PA, Lindsay P, Thomas DG, Rutter GA, Shi P, Rossiter P, Pizey MH (2011) Passive treatment of acid mine drainage using waste mussel shell, Stockton coal mine, New Zealand. In Proc: Proc. 7th Australian Acid and Metalliferous Drainage Workshop, Darwin, Australia, 21-24 June 2011.

Cubillas P, Köhler S, Prieto M, Causserand C, Oelkers EH (2005a) How do mineral coatings affect dissolution rates? An experimental study of coupled CaCO3 dissolution—CdCO3 precipitation. Geochim Cosmochim Acta 69 (23):5459-5476.

Cubillas P, Köhler S, Prieto M, Chaïrat C, Oelkers EH (2005b) Experimental determination of the dissolution rates of calcite, aragonite, and bivalves. Chem Geol 216 (1–2):59-77.

Das BK, Mandal SM, Bhattachasrya J (2012) Understanding of the biochemical events in a chemo-bioreactor during continuous acid mine drainage treatment. Environmental Earth Sciences 66 (2):607-614.

Das BM (2002) Permeability. In: Principles of Geotechnical Engineering. 6. Brooks Cole/Thompson Learning, Pacific Grove, pp 143-146

Daubert LN, Brennan RA (2007) Passive Remediation of Acid Mine Drainage Using Crab Shell Chitin. Environmental Engineering Science 24 (10):1475-1480.

Davies H, Weber P, Lindsay P, Craw D, Pope J (2011) Characterisation of acid mine drainage in a high rainfall mountain environment, New Zealand. Sci Total Environ 409 (15):2971-2980.

Dold B (2003) Basic concepts of environmental geochemistry of sulfide mine-waste. University of Lausanne

Dold B (2006) Element flows associated with marine shore mine tailings deposits. Environmental Science and Technology 40:752-758.

Drury WJ (1999) Treatment of Acid Mine Drainage with Anaerobic Solid-Substrate Reactors. Water Environ Res 71 (6):1244-1250.

Dufresne K, Neculita CM, Brisson J, Genty T (2015) Metal retention mechanisms in pilot-scale constructed wetlands receiving acid mine drainage. In Proc: 10th International Conference on Acid Rock Drainage & International Mine Water Association Annual Conference, Santiago, Chile, 2015. IMWA, pp 1-6.

Dundar MS, Altundag H, Eyupoglu V, Keskin SC, Tutunoglu C (2011) Determination of heavy metals in lower Sakarya river sediments using a BCR-sequential extraction procedure. Environ Monit Assess 184 (1):33-41.

Dvorak DH, Hedin RS, Edenborn HM, McIntire PE (1992) Treatment of metal-contaminated water using bacterial sulfate-reduction: Results from pilot-scale reactors Biotechnol Bioeng 40 (5):609-616.

Dyer JA, Trivedi P, Scrivner NC, Sparks DL (2004) Surface complexation modeling of zinc sorption onto ferrihydrite. J Colloid Interface Sci 270 (1):56-65.

Eger P, Wagner J (2002) Wetland Treatment Systems - How Long Will They Really Work? Mining and the Environment:14.

Ehrlich HL, Newman DK (2008a) Geomicrobial Processes: Physiological and Biochemical Overview. In: Geomicrobiology. 6, 5th edn. CRC Press, pp 89-116

Ehrlich HL, Newman DK (2008b) Geomicrobiology of Iron. In: Geomicrobiology, 5th Edition. Chapter 16. CRC Press, pp 279-346

Emmanuel S, Levenson Y (2014) Limestone weathering rates accelerated by micron-scale grain detachment. Geology

Ettner DC (1999) Pilot scale constructed wetland for the removal of nickel from tailings drainage, Southern Norway. In Proc: Congress of the International Mine Water Association, Sevilla, Spain, 13-17 September 1999. pp 207-211.

Farag S, Das R, Strosnider WH, Wilson RT (2015) Possible health effects of living in proximity to mining sites near potosi, Bolivia. J Occup Environ Med 57 (5):543-551.

Figueroa L, Miller A, Zaluski M, Bless D (2007) Evaluation of a two-stage passive treatment approach for mining-influenced waters. In Proc: National Meeting of the American Society of Mining and Reclamation Gillette, WY, 2007. ASMR, pp 238-247.

Gandy C, Jarvis A (2012) The Influence of Engineering Scale and Environmental Conditions on the Performance of Compost Bioreactors for the Remediation of Zinc in Mine Water Discharges. Mine Water and the Environment 31 (2):82-91.

Gibert O, De Pablo J, Cortina JL, Ayora C (2002) Treatment of acid mine drainage by sulphate-reducing bacteria using permeable reactive barriers: A review from laboratory to full-scale experiments. Re/Views in Environmental Science & Bio/Technology 1:327-333.

Gibert O, De Pablo J, Cortina JL, Ayora C (2003) Evaluation of municipal compost/limestone/iron mixtures as filling material for permeable reactive barriers for in-situ acid mine drainage treatment. J Chem Technol Biotechnol 78 (5):489-496.

Gibert O, de Pablo J, Cortina JL, Ayora C (2004) Chemical characterisation of natural organic substrates for biological mitigation of acid mine drainage. Water Res 38 (19):4186-4196.

Gibert O, De Pablo J, Cortina JL, Ayora C (2005) Municipal compost-based mixture for acid mine drainage bioremediation: Metal retention mechanisms. Appl Geochem 20 (9):1648-1657.

Goetz ER, Riefler RG (2014) Performance of steel slag leach beds in acid mine drainage treatment. Chem Eng J 240:579-588.

Gusek JJ (2002) Sulphate-reducing bioreactor design and operating issues: Is this the passive treatment technology for your mine drainage? In Proc: National Meeting of the American Society of Mining and Reclamation, Park City (UT), 15-18 Sept 2002.

Gusek JJ (2004) Scalling up design challenges for large scale sulfate reducing bioreactors. In Proc: National Meeting of the American Society of Mining and Reclamation and the 25th West Virginia Surface Mine Drainage Task Force, Morgantown, WV, 2004. ASMR, pp 752-765.

Gusek JJ (2005) Design challenges for large scale sulfate-reducing bioreactors. In: Calabrese EJ, Kostecki PT, Dragun J (eds) Contaminated Soils, Sediments and Water, vol 9. 4. Springer US, pp 33-44

Gusek JJ (2008) Passive Treatment 101: An Overview of the Technologies. Paper presented at the 2008 U.S. EPA/National Groundwater Association's Remediation of Abandoned Mine Lands Conference, Denver, CO, October 2-3, 2008

Gusek JJ (2013) A Periodic Table of Passive Treatment for Mining Influenced Water - Revisited. In Proc: International Mine Water Association, Golden CO, USA, 2013. Reliable Mine Water Technology. Publication Printers, pp 575-581.

Gusek JJ, Wildeman TR (2002a) A new millenium of passive treament systemsof acid rock drainage: advances in design and construction since 1988. In Proc: National Meeting of the American Society for Mining and Reclamation, Lexington, Kentucky, 9-13 June 2002a. ASMR, pp 935-951.

Gusek JJ, Wildeman TR (2002b) Passive Treatment of Aluminum-Bearing Acid Rock Drainage. In Proc: 23rd Annual West Virginia Surface Mine Drainage Task Force Symposium, Morgantown, WV, 16-17 April 2002b. pp 1-9.

Harding JS (2005) Impacts of metals on stream communities. In: Moore TA, Black A, Centeno JA, Harding JS, Trumm D (eds) Metal contaminants in New Zealand: source, treatments, and effects on ecology and huma health. Resolutionz Press,

Hass A, Fine P (2010) Sequential Selective Extraction Procedures for the Study of Heavy Metals in Soils, Sediments, and Waste Materials—a Critical Review. Crit Rev Environ Sci Technol 40 (5):365-399.

Heal KV, Salt CA (1999) Treatment of acidic metal-rich drainage from reclaimed ironstone mine spoil. Water Sci Technol 39 (12):141-148.

Hedin RS, Nairn RW, Kleinmann RLP (1994) Passive treatment of coal mine drainage. U.S. Department of Interior. Bureau of Mines. Information Circular 9389.

Hendryx M, Fulk F, McGinley A (2012) Public Drinking Water Violations in Mountaintop Coal Mining Areas of West Virginia, USA. Water Quality, Exposure and Health 4 (3):169-175.

Herbert RB, Benner SG, Blowes DW (2000) Solid-phase iron-sulfur geochemistry of a reactive barrier for treatment of mine drainage. Appl Geochem 15:1331-1343.

Herbert RB, Benner SG, Pratt AR, Blowes DW (1998) Surface chemistry and morphology of poorly crystalline iron sulfides precipitated in media containing sulfate-reducing bacteria. Chem Geol 144:87-97.

Higgins J, Whitford J, Mattes A (2003) The use of enginnered wetlands to treat acid mine drainage. Paper presented at the German-North American Environmental Conference: The Rehabilitation of Industrial Wasteland and Post-Mining Landscapes, Görlitz, Saxony,

Hill JW, Petrucci RH, McCreary TW, Perry SS (2004) General Chemistry. 4th edn. Prentice Hall, Upper Saddle River (NJ)

Hill RJ (2014) Environmental Catalogue Version 9.0.

Hogsden KL, Harding JS (2011) Consequences of acid mine drainage for the structure and function of benthic stream communities: a review. Freshwater Science 31 (1):108-120.

Hua G, Zeng Y, Zhao Z, Cheng K, Chen G (2014) Applying a resting operation to alleviate bioclogging in vertical flow constructed wetlands: an experimental lab evaluation. J Environ Manage 136 (1):47-53.

Huisman JL, Schouten G, Schultz C (2006) Biologically produced sulphide for purification of process streams, effluent treatment and recovery of metals in the metal and mining industry. Hydrometallurgy 83 (1-4):106-113.

Hullebusch E, Utomo S, Zandvoort M, Llens P (2005) Comparison of three sequential extraction procedures to describe metal fractionation in anaerobic granular sludges. Talanta 65 (2):549-558.

Hupfer M, Zak D, Rossberg R, Herzog C, Pothig R (2009) Evaluation of a well-established sequential phosphorus fractionation technique for use in calcite-rich lake sediments: identification and prevention of artifacts due to apatite formation. Limnol Oceanogr Methods 7:399-410.

Hutchinson R, Nairn RW (2005) Determining hydraulic conductivities in a vertical flow system. In Proc: National Meeting of the American Society for Mining and Reclamation, Breckinridge, CO, 19-24 June 2005. ASMR, pp 504-511.

ITRC, Interstate Technology & Regulatory Council (2012) Biochemical Reactors for Mining-Influenced Waste. (www.itrcweb.org), Washington, D.C.

James TI (2003) Water quality of stream draining various coal measures in the North-Central West Coast. In Proc: Australasian Institute of Mining and Metalurgy, New

Zealand Branch Annual Conference, Greymouth, NZ, 3-5 September 2003. p not paginated.

Johnson DB, Hallberg KB (2005a) Acid mine drainage remediation options: a review. Sci Total Environ 338 (1-2):3-14.

Johnson DB, Hallberg KB (2005b) Biogeochemistry of the compost bioreactor components of a composite acid mine drainage passive remediation system. Sci Total Environ 338 (1-2):81-93.

Jong T, Parry DL (2003) Removal of sulfate and heavy metals by sulfate reducing bacteria in short-term bench scale upflow anaerobic packed bed reactor runs. Water Res 37 (14):3379-3389.

Jong T, Parry DL (2004a) Adsorption of Pb(II), Cu(II), Cd(II), Zn(II), Ni(II), Fe(II), and As(V) on bacterially produced metal sulfides. J Colloid Interface Sci 275 (1):61-71.

Jong T, Parry DL (2004b) Heavy metal speciation in solid-phase materials from a bacterial sulfate reducing bioreactor using sequential extraction procedure combined with acid volatile sulfide analysis. J Environ Monit 6 (4):278-285.

Jurjovec J, Ptacek CJ, Blowes DW (2002) Acid neutralization mechanisms and metal release in mine tailings: A laboratory column experiment. Geochim Cosmochim Acta 66 (9):1511-1523.

Kadlec RH, Wallace SD (2009) Treatment Wetlands. 2nd edn. CRC Press, Boca Raton, FL

Kaksonen AH, Puhakka JA (2007) Sulfate Reduction Based Bioprocesses for the Treatment of Acid Mine Drainage and the Recovery of Metals. Eng Life Sci 7 (6):541-564.

Kalin M (2004) Passive mine water treatment: the correct approach? Ecol Eng 22 (4-5):299-304.

Kalin M, Fyson A, Wheeler WN (2006) The chemistry of conventional and alternative treatment systems for the neutralization of acid mine drainage. Sci Total Environ 366 (2-3):395-408.

Kerndorff H, Schnitzer M (1980) Sorption of metals on humic acid. Geochim Cosmochim Acta 44 (11):1701-1708.

Kirby CS, Cravotta CA (2005) Net alkalinity and net acidity 1: Theoretical considerations. Appl Geochem 20 (10):1920-1940.

Kolmert A, Johnson DB (2001) Remediation of acidic waste waters using immobilised, acidophilic sulfate-reducing bacteria. J Chem Technol Biotechnol 76:836-843.

Kuyucak N, Chabot F, Martschuk J (2006) Successful implementation and operation of a passive treatment system in an extremely cold climate, northern Quebec, Canada. In Proc: 7th International Conference on Acid Rock Drainage (ICARD), St Louis (MO), 2006. American Society of Mining and Reclamation (ASMR).

Lebron I, Suarez DL (1999) Mechanisms and Precipitation Rate of Rhodochrosite at 25°C as Affected by PCO2 and Organic Ligands. Soil Sci Soc Am J 63 (3):561-568.

Ledin M, Pedersen K (1996) The environmental impact of mine wastes: Roles of microorganisms and their significance in treatment of mine wastes. Earth-Sci Rev 41 (1–2):67-108.

Lo IM-C, Yang X-Y (1998) Removal and redistribution of metals from contaminated soil by a sequential extraction method. Waste Manage 18:1-7.

Logan MV, Ahmann DM, Figueroa LA (2003) Assessment of microbial activity in anaerobic columns treating synthetic mine drainage. In Proc: National Meeting of the American Society of Mining and Reclamation and The 9th Billings Land Reclamation Symposium, Billings, (MO), 3-6 June 2003 2003. American Society of Mining and Reclamation, Lexington (KY), pp 658-678.

Logan MV, Reardon KF, Figueroa LA, McLain JET, Ahmann DM (2005) Microbial community activities during establishment, performance, and decline of bench-scale passive treatment systems for mine drainage. Water Res 39 (18):4537-4551.

Lyew D, J.D. S (1997) Effects of physical parameters of a gravel bed on the activity of sulphate-reducing bacteria in the presence of acid mine drainage. J Chem Technol Biotechnol 70:223-230.

Machemer SD, Reynolds JS, Laudon LS, Wildeman TR (1993) Balance of S in a constructed wetland built to treat acid mine drainage, Idaho Springs, Colorado, U.S.A. Appl Geochem 8 (6):587-603.

Machemer SD, Wildeman TR (1992) Adsorption compared with sulfide precipitation as metal removal processes from acid mine drainage in a constructed wetland. J Cont Hydro 9 (1–2):115-131.

Mayes WM, Batty LC, Younger PL, Jarvis AP, Kõiv M, Vohla C, Mander U (2009) Wetland treatment at extremes of pH: A review. Sci Total Environ 407 (13):3944-3957.

McCauley CA (2011) Assessment of passive treatment and biochemical reactors for ameliorating acid mine drainage at Stockton coal mine. PhD thesis, University of Canterbury, Christchurch, New Zealand

McCauley CA, O'Sullivan AD, Milke MW, Weber PA, Trumm DA (2009) Sulfate and metal removal in bioreactors treating acid mine drainage dominated with iron and aluminum. Water Res 43 (4):961-970.

McCauley CA, Weber PA, Trumm DA, O'Sullivan AD (2008) Performance of mesocosm-scale sulfate-reducing bioreactors for treating acid mine drainage in New Zealand. In Proc: National Meeting of the American Society of Mining and Reclamation, New Opportunities to Apply Our Science., Richmond, VA, USA, June 14-19 2008. ASMR.

MfE (2004) Hazardous Waste Guidelines: Landfill Waste Acceptance Criteria and Landfill Classification. Ministry for the Environment (www.mfe.govt.nz), Wellington, New Zealand

Mills AL (1999) The Role of Bacteria in Environmental Geochemistry. In: Plumlee GS, Logsdon MJ (eds) The Environmental Geochemistry of Mineral Deposits. Part A: Processes, Techniques and Health Issues., vol 6A. Chapter 5. Society of Economic Geologists, INC., pp 125-132

Morse JW, Arvidson RS (2002) The dissolution kinetics of major sedimentary carbonate minerals. Earth-Sci Rev 58 (1-2):51-84.

Neculita CM (2010) Mine-impacted water treatment – how the bottom line can be raised? International Journal of Mining, Reclamation and Environment 24 (3):193-194.

Neculita CM, Zagury GJ (2008) Biological treatment of highly contaminated acid mine drainage in batch reactors: Long-term treatment and reactive mixture characterization. J Hazard Mater 157 (2–3):358-366.

Neculita CM, Zagury GJ, Bussière B (2007a) Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: Critical review and research needs. Journal of Environmental Quality 36 (1):1-16.

Neculita CM, Zagury GJ, Bussière B (2008a) Effectiveness of sulfate-reducing passive bioreactors for treating highly contaminated acid mine drainage: I. Effect of hydraulic retention time. Appl Geochem 23 (12):3442-3451.

Neculita CM, Zagury GJ, Bussière B (2008b) Effectiveness of sulfate-reducing passive bioreactors for treating highly contaminated acid mine drainage: II. Metal removal mechanisms and potential mobility. Appl Geochem 23 (12):3545-3560.

Neculita CM, Zagury GJ, Kulnieks VI (2007b) Short-term and long-term bioreactors for acid mine drainage treatment. Paper presented at the Annual International Conference on Soils, Sediments, Water and Energy, MA, USA,

Newcombe C, Brennan R (2009) Improved Passive Treatment of Acid Mine Drainage in Mushroom Compost Amended with Crab-Shell Chitin. J Environ Eng 136 (6):616-626.

Niyogi DK, Harding JS, Simon KS (2013) Organic matter breakdown as a measure of stream health in New Zealand streams affected by acid mine drainage. Ecol Indicators 24:510-517.

Nordstrom DK (1982) The effect of sulfate on aluminum concentration in natural waters: some stability relation in the system Al_2O_3-SO_3-H_2) at 298 K. Geochim Cosmochim Acta 46:681-692.

Nordstrom DK (2009) Acid rock drainage and climate change. Journal of Geochemical Exploration 100 (2–3):97-104.

Nordstrom DK (2011a) Hydrogeochemical processes governing the origin, transport and fate of major and trace elements from mine wastes and mineralized rock to surface waters. Appl Geochem 26 (11):1777-1791.

Nordstrom DK (2011b) Mine waters: acidic to circmneutral. Elements 7 (6):393-398.

Nordstrom DK, Alpers CN (1999a) Geochemistry of acid mine waste. In: Plumlee GS, Logsdon MJ (eds) The Environmental Geochemistry of Mineral Deposits. Part A: Processes, Techniques and Health Issues. Reviews in Economic Geology., vol 6A. Chapter 6. Society of Economic Geologists, INC., pp 133-160

Nordstrom DK, Alpers CN (1999b) Negative pH, efflorescent mineralogy, and consequences for environmental restoration at the Iron Mountain Supefund site, California. In Proc: National Academy of Sciences colloquium "Geology, Mineralogy, and Human Welfare", 1999b. pp 3455–3462.

Nordstrom DK, Ball JW (1986) The Geochemical Behavior of Aluminum in Acidified Surface Waters. Science 232 (4746):54-56.

Nordstrom DK, Blowes DW, Ptacek CJ (2015) Hydrogeochemistry and Microbiology of Mine Drainage: An Update. Appl Geochem

O'Sullivan AD (2005) Passive treatment technologies for managing metal mine wastes: lessons learnt from global applications. In: Moore TA, Black A, Centeno JA, Harding JS, Trumm D (eds) Metal contaminants in New Zealand: source, treatments, and effects on ecology and huma health. 14. Resolutionz Press, Christchurch, pp 195-215

Okabe S, Nielsen PH, Characklis WG (1992) Factors affecting microbial sulfate reduction by Desulfovibrio desulfuricans in continuous culture: Limiting nutrients and sulfide concentration. Biotechnol Bioeng 40 (6):725-734.

Piatak NM, Seal RR, Sanzolone RF, Lamothe PJ, Brown ZA (2006) Preliminary Results of Sequential Extraction Experiments for Selenium on Mine Waste and Stream Sediments in Vermont, Maine, and New Zealand. U.S. Geological Survey. Open-File Report 2006-1184

Piatak NM, Seal RR, Sanzolone RF, Lamothe PJ, Brown ZA, Adams M (2007) Sequential Extraction Results and Mineralogy of Mine Waste and Stream Sediments Associated with Metal Mines in Vermont, Maine, and New Zealand. US Geological Survey. Open-File Report 2007-1063

PIRAMID (2003) Engineering guidelines for the passive remediation of acidic and/or metalliferous mine drainage and similar wastewaters. University of Newcastle Upon Tyne.

Place DL, Claveau E, Figueroa LA (2005) Tracking organic substrate alteration in passive reactive zones for planning and monitoring. In Proc: National Meeting of the American Society of Mining and Reclamation, 19-25 June, 2005 2005. American Society of Mining and Reclamation (ASMR), pp 921-934.

Place DL, Figueroa LA, Wildeman TR, Reisman DJ (2006) Characterizing and tracking reactive mixture alterations: new tools for passive treatment system design and monitoring. In Proc: 7th International Conference on Acid Rock Drainage (ICARD), St. Louis (MO), 2006. American Society of Mining and Reclamation, pp 1605-1619.

Plumlee GS (1999) The Environmental Geochemistry of Mineral Deposits. In: Plumlee GS, Logsdon MJ (eds) The Environmental Geochemistry of Mineral Deposits. Part A: Processes, Techniques and Health Issues. Reviews in Economic Geology., vol 6. pp 71-116

Pope J, Newman N, Craw D, Trumm D, Rait R (2010) Factors that influence coal mine drainage chemistry West Coast, South Island, New Zealand. N Z J Geol Geophys 53 (2):115-128.

Pósfai M, Dunin-Borkowski RE (2006) Sulfides in Biosystems. In: Vaughan DJ (ed) Sulfide Mineralogy and Geochemistry, vol 61. Reviews in Mineralogy and Geochemistry, 13. pp 679-714

Post JE (1999) Manganese oxides minerals: crystal structures and economic and environmental significance. Proc Natl Acad Sci USA 96:3447-3454.

Postgate JR (1979) The sulphate-reducing bacteria. 1st edn. Cambridge University Press, Cambridge

Powrie W (2004) Soil Mechanics: Concepts and Applications. 2nd edn. CRC Press, London

Prasad D, Wai M, Bérubé P, Henry JG (1999) Evaluating Substrates in the Biological Treatment of Acid Mine Drainage. Environ Technol 20 (5):449-458.

Pruden A, Pereyra LP, Hiibel SR, Inman LY, Kashani N, Reardon KF, Reisman DJ (2006) Microbiology of sulfate-reducing passive treatment systems. Paper presented at the 7th International Conference on Acid Rock Drainage (ICARD), St-Louis (MO),

Rao CRM, Sahuquillo A, Lopez Sanchez JF (2007) A Review of the Different Methods Applied in Environmental Geochemistry For Single and Sequential Extraction of Trace Elements in Soils and Related Materials. Water Air Soil Pollut 189 (1-4):291-333.

Reis MAM, Almeida JS, Lemos PC, Carrondo MJT (1992) Effect of hydrogen-sulfide on growth of sulfate reducing bacteria. Biotechnol Bioeng 40 (5):593-600.

Reisman D, Rutkowski T, Smart P, Gusek JJ (2008) Construction and instrumentation of a pilot treatment system at the Standard Mine Superfound, Crested Butte, CO. In Proc: National Meeting of the American Society of Mining and Reclamation, Lexington, KY, 2008. ASMR, pp 892-909.

Robinson-Lora MA, Brennan RA (2009) Efficient metal removal and neutralization of acid mine drainage by crab-shell chitin under batch and continuous-flow conditions. Bioresour Technol 100 (21):5063-5071.

Robinson-Lora MA, Brennan RA (2010a) Biosorption of manganese onto chitin and associated proteins during the treatment of mine impacted water. Chem Eng J 162 (2):565-572.

Robinson-Lora MA, Brennan RA (2010b) The role of precipitation and biosorption in the abiotoc removal of manganese with crab-shell chitin. In Proc: National Meeting of the American Society of Mining and Reclamation, Pittsburgh, 5-11 June 2010 2010b. ASMR, pp 828-846.

Rose AW (2010) Advances in passive treatment of coal mine drainage (1998-2009). Paper presented at the National Meeting of the American Society of Mining and Reclamation, Pittsburgh, PA, 5-11 June 2010

Rose AW, Dietz JM (2002) Case studies of passive treatment systems: Vertical flow systems. In Proc: National Meeting of the American Society of Mining and Reclamation, KY, USA, 2002. American Society of Mining and Reclamation (ASMR), pp 776-797.

Rose AW, Morrow T, Dunn M, Denholm C (2007) Mode of gypsum precipitation in vertical flow ponds. In Proc: National Meeting of the American Society of Mining and Reclamation, Gillette, WY, 2007.

Rossman W, Wytovitch E, Seif JM (1997) Abandoned mines-Pennsylvania's single biggest water pollution problem. Pennsylvania Department of Environmental Protection, Harrisburg (PA)

Ruiz F, Borrego J, González-Regalado ML, López González N, Carro B, Abad M (2008) Impact of millennial mining activities on sediments and microfauna of the Tinto River estuary (SW Spain). Mar Pollut Bull 56 (7):1258-1264.

Sahinkaya E, Gungor M (2010) Comparison of sulfidogenic up-flow and down-flow fluidized-bed reactors for the biotreatment of acidic metal-containing wastewater. Bioresour Technol 101 (24):9508-9514.

Sheoran AS, Sheoran V, Choudhary RP (2010) Bioremediation of acid-rock drainage by sulphate-reducing prokaryotes: A review. Min Eng 23 (14):1073-1100.

Simmons J, Ziemkiewicz P, Black DC (2001) Use of steel slag leach beds for the treatment of acid mine drainage: the McCarty highwall project. Paper presented at the National Association of Abandoned Mine Lands Annual Conference, Athens, OH, 19-22 August 2001

Skousen JG, Rose AW, Geidel G, Foreman J, Evans R, Hellier W (1998) Handbook of Technologies for Avoidance and Remediation of Acid Mine Drainage. ACID DRAINAGE TECHNOLOGY INITIATIVE (ADTI), Morgantown (WV)

Skousen JG, Sexstone A, Ziemkiewiecz PF (2000) Acid mine drainage controle and treatment. In: Barnhisel RI, Darmody RG, Daniels WL (eds) Reclamation of Drastically Disturbed Lands. 1106. American Society of Agronomy-Crop Science Society of America-Soil Science Society of America,

Sobolewski A (1999) A Review of Processes Responsible for Metal Removal in Wetlands Treating Contaminated Mine Drainage. Int J Phytoremediation 1 (1):19-51.

Stumm W, Morgan JJ (1996) Aquatic chemistry: chemical equilibria and rates in natural waters. Wiley-Interscience, New York

Swash PM, Monhemius AJ (2005) Characteristics and stabilities of residues from the Wheal Jane constructed wetlands. Sci Total Environ 338 (1-2):95-105.

Tang K, Baskaran V, Nemati M (2009) Bacteria of the sulphur cycle: An overview of microbiology, biokinetics and their role in petroleum and mining industries. Biochem Eng J 44 (1):73-94.

Tessier A, Campbell PGC, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry 51 (7):844-851.

Thomas RC, Romanek CS (2002a) Acid rock drainage in a vertical flow wetland I: Acidity neutralization and alkalinity generation. In Proc: National Meeting of the American Society of Mining and Reclamation, Lexington, KY, 9-13 June 2002 2002a. ASMR, 3134 Montavesta Rd., Lexington, KY 40502.

Thomas RC, Romanek CS (2002b) Passive treatment of low-pH, ferric iron-dominated acid rock drainage in a vertical flow wetland II: Metal removal. In Proc: National Meeting of the American Society of Mining and Reclamation, Lexington, KY, 9-13 June 2002 2002b. ASMR, 3134 Montavesta Rd., Lexington, KY 40502.

Trumm D (2007) Acid Mine Drainage in New Zealand. Reclamation Matters (1):23-28.

Trumm D (2010) Selection of active and passive treatment systems for AMD—flow charts for New Zealand conditions. N Z J Geol Geophys 53 (2-3):195-210.

Trumm D, Ball J (2014) Use of sulfate-reducing mussel shell reactors in New Zealand for treatment of acid mine drainage. Paper presented at the West Virginia Mine Drainage Task Force Symposium, Morgantown, WV, 25-26 March

Tsukamoto TK, Killion HA, Miller GC (2004) Column experiments for microbiological treatment of acid mine drainage: low-temperature, low-pH and matrix investigations. Water Res 38 (6):1405-1418.

URS (2003) Passive and semi-active treatment of acid rock drainage from metal mines-state of the practice. Prepared for U.S. Army Corps of Engineers, Concord, Massachusetts.

US-EPA (1980) Control and Treatment Technology for the Metal Finishing Industry: Sulfide precipitation, (625/8-80-003), Cincinnati, OH

US-EPA (2000) Coal Remining Best Management Practices Guidance Manual, (EPA 821-R-00-007), Washington DC

US-EPA (2001) Method 1684: Total, Fixed and Volatile Solids in Water, Solids and Biosolids, (EPA-821-R-01-015), Washington DC

Utgikar VP, Chen B-Y, Tabak HH, Bishop DF, Govind R (2000) Treatment of acid mine drainage: I. Equilibrium biosorption of zinc and copper on non-viable activated sludge. Int Biodeterior Biodegrad 46 (1):19-28.

Utgikar VP, Harmon SM, Chaudhary N, Tabak HH, Govind R, Haines JR (2002) Inhibition of sulfate-reducing bacteria by metal sulfide formation in bioremediation of acid mine drainage. Environ Toxicol 17 (1):40-48.

Van Damme PA, Hamel C, Ayala A, Bervoets L (2008) Macroinvertebrate community response to acid mine drainage in rivers of the High Andes (Bolivia). Environ Pollut 156 (3):1061-1068.

Venot C, Figueroa L, Brennan RA, Wildeman TR, Reisman D, Sieczkowski M (2008) Comparing Chitin and Organic Substrates on the National Tunnel Waters in Blackhawk, Colorado for Manganese removal. In Proc: National Meeting of the American Society of Mining and Reclamation, New Opportunities to Apply Our Science, Richmond, VA, June 14-19 2008. ASMR, pp 1352-1366.

Waller PA, Pickering WF (1992) Effect of time and pH on the lability of copper and zinc sorbed on humic acid particles. Chem Spec Bioavail 4 (1):29-41.

Wallmann K, Kersten M, Gruber J, Förstner U (1993) Artifacts in the Determination of Trace Metal Binding Forms in Anoxic Sediments by Sequential Extraction. Int J Environ Anal Chem 51 (1-4):187-200.

Wang C, Zhang Y, Li H, Morrison RJ (2013) Sequential extraction procedures for the determination of phosphorus forms in sediment. Limnology 12 (2):147-157.

Watzlaf GR, Schroeder KL, Kleinmann RLP, Kairies CL, Nairn RW (2004) The Passive Treatment of Coal Mine Drainage. National Energy Technology Laboratory, US Department of Energy.

Waybrant KR, Blowes DW, Ptacek CJ (1998) Selection of reactive mixtures for use in permeable reactive walls for treatment of mine drainage. Environ Sci Technol 32 (13):1972-1979.

Waybrant KR, Ptacek CJ, Blowes DW (2002) Treatment of Mine Drainage Using Permeable Reactive Barriers: Column Experiments. Environ Sci Technol 36 (6):1349-1356.

Webster-Brown JG, Dee TJ, Hegan AF (2012) Metal removal via particulate material in a lowland river system. Water Sci Technol 66 (7):1439-1445.

Webster JG, Swedlund PJ, Webster KS (1998) Trace metal adsorption onto an acid mine drainage iron(III) (oxy)hydroxysulfate. Environ Sci Technol 32 (10):1361-1368.

White C, Gadd GM (1996) A comparison of carbon/energy and complex nitrogen sources for bacterial sulphate-reduction: potential applications to bioprecipitation of toxic metals as sulphides. Journal of Industrial Microbiology 17 (2):116-123.

Whitehead PG, Hall G, Neal C, Prior H (2005) Chemical behaviour of the Wheal Jane bioremediation system. Sci Total Environ 338 (1-2):41-51.

Wicke D, Cochrane TA, O'Sullivan A (2012) Build-up dynamics of heavy metals deposited on impermeable urban surfaces. J Environ Manage 113:347-354.

Wieder RK (1989) A survey of constructed wetlands for acid coal mine drainage treatment in the Eastern United States. Wetlands 9 (2):299-315.

Wieder RK, Lang GE (1982) Modification of acid mine drainage in a freshwater wetland. In Proc: Symposium on Wetlands of the Unglaciated Appalachian Region, Morgantown (WV), 1982. West Virginia University Press.

Wieder RK, Lang GE (1984) Influence of wetlands and coal mining on stream water chemistry. Water Air Soil Pollut 23:381-396.

Wildeman TR, Brodie GA, Gusek JJ (1993) Wetland Design for Mining Operations. BiTech Publishing Co., Vancouver, B.C. Canada

Wildeman TR, Gusek JJ, Higgins J (2006) Passive treatment of mine influenced waters. In: Course Material for the ARD Treatment Short Course presented at the 7th International Conference on Acid Rock Drainage (ICARD). St. Louis (MI) 26-30 March 2006

Willow MA, Cohen RRH (2003) pH, dissolved oxygen, and adsorption effects on metal removal in anaerobic bioreactors. Journal of Environmental Quality 32 (4):1212-1221.

Yoo K, Sasaki K, Hiroyoshi N, Tsunekawa M, Hirajima T (2004) The effect of Mn2+ concentration on Mn removal by a sulfate reducing bacteria bioreactor. Materials transactions 45 (7):2429-2434.

Younger PL (2004) The Mine Water Pollution Threat to Water Resources and Its Remediation in Practice. IDS Water Europe

Younger PL, Banwart SA, Hedin RS (2002) Mine water: Hydrology, Pollution, Remediation. Environmental Pollution. Kluwer Academic Publishers, Dordrecht

Zagury GJ, Colombano SM, Narasiah KS, Ballivy G (1997) Neutralization of Acid Mine Tailings by Addition of Alkaline Sludges from Pulp and Paper Industry. Environ Technol 18 (10):959-973.

Zagury GJ, Kulnieks VI, Neculita CM (2006) Characterization and reactivity assessment of organic substrates for sulphate-reducing bacteria in acid mine drainage treatment. Chemosphere 64 (6):944-954.

Zagury GJ, Neculita CM, Bussière B (2005) Passive biological treatment of acid mine drainage: Challenges of the 21st century. Paper presented at the 2nd Symposium on Mining and the Environment, Rouyn-Noranda (Canada), 15-18 Mai 2005

Zhou J-M, Dang Z, Cai M-F, Liu C-Q (2007) Soil Heavy Metal Pollution Around the Dabaoshan Mine, Guangdong Province, China. Pedosphere 17 (5):588-594.

Ziemkeivic PF, Skousen JG, Simmons J (2003) Long term performance of passive acid mine drainage treatment systems. Mine Water and the Environment 22:118-129.

Zipper CE, Skousen JG (2010) Influent Water Quality Affects Performance of Passive Treatment Systems for Acid Mine Drainage. Mine Water and the Environment 29 (2):135-143.

Zobrist J, Giger W (2013) Mining and the environment. Environmental Science and Pollution Research 20 (11):7487-7489.